COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). in the name of:
Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY:
Ludwig Institute for Cancer Research under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, **John Stanley MATTICK,** Professor of Molecular Biology, and Director of the Australian Research Council's Special Research Centre for Functional and Applied Genomics, Director of the Australian Genome Research Facility, and Co-Director of the Institute for Molecular Bioscience at the University of Queensland, St Lucia, Queensland 4072, Australia, declare as follows:

1 BACKGROUND and QUALIFICATIONS

1.1 I am currently the Foundation Professor of Molecular Biology in the Department of Biochemistry at The University of Queensland, the Director of the ARC Special Research Centre for Functional and Applied Genomics, the Director of the Australian Genome Research Facility, and the Co-Director of the Institute for Molecular Bioscience at The University of Queensland in Brisbane, Australia.

1.2 The research that I have conducted over the last 28 years is presented in my curriculum vitae through my publications and presentation. Exhibited to me and marked with the letters "**JSM-1**" is a copy of my curriculum vitae, which itemises the publications and presentations of which I have been an author or co-author.

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1.3 I received my B.Sc with First Class Honours in Biochemistry from the University of Sydney in 1972, followed by a PhD in Biochemistry in 1977 from Monash University, on the topic of mitochondrial DNA replication and mutation. I then undertook postdoctoral work on the molecular biology of the fatty acid synthetase complex at the Baylor College of Medicine in Houston Texas from 1977 to 1981. My work on the architecture and function of this complex is now standard in biochemistry textbooks. I returned to Australia in early 1981 to work as a research scientist at the (then) CSIRO Division of Molecular Biology in Sydney, where I was the leader of a team responsible for the development of one of the world's first genetically engineered vaccines (against bovine footrot), for which I was awarded the 1989 Pharmacia-LKB Biotechnology Medal from the Australian Biochemical Society.

1.4 In 1988, I was appointed the Foundation Professor of Molecular Biology and Foundation Director of the Centre for Molecular Biology and Biotechnology at the University of Queensland. The Centre was subsequently made a Special Research Centre of the Australian Research Council, and in 1994 was renamed the ARC Special Research Centre for Molecular and Cellular Biology. The Centre grew rapidly and developed a strong national and international reputation for its research on the molecular biology of mammals and their diseases, including gene mapping, gene regulation, and developmental and cell biology.

1.5 In 1988, I was appointed the Director of the Australian Genome Research Facility that was established under the aegis of the Australian Government's Major National Research Facilities Program at the University of Queensland in Brisbane and at the Walter and Eliza Hall Institute for Medical Research in Melbourne.

1.6 In 2000, the Centre for Molecular and Cellular Biology amalgamated with the Centre for Drug Design and Development, and elements of other research centres, notably the Centre for Microscopy and Microanalysis and the Advanced Computational Modelling Centre, to form a new Institute for Molecular Bioscience, which is currently being constructed at the University of Queensland. I am Co-Director and Research Director of the Institute for Molecular Bioscience.

1.7 Also in 2000 I and my colleagues successfully applied for a new Special Research Centre grant from the Australian Research Council, and I now

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also hold the position of Director of the ARC Special Research Centre for Functional and Applied Genomics, within the Institute for Molecular Bioscience. This Centre and the Institute is concerned with integrated research ranging across the spectrum from genomics and genetics, developmental biology, cell biology, structural biology, and biological chemistry, all underpinned by advanced bioinformatics and computational biology.

1.8 Now exhibited to me and marked with the letters "**JSM-2**" is a summary of the research that I have conducted over the last 29 years. For ease of description I have not identified, in the summary, all of the publications that I have authored or co-authored. Instead, I have identified only those publications that I consider are my most significant. I refer to those publications by the numbers corresponding to those publications set out in my curriculum vitae.

1.9 I have been intimately involved in molecular biology since 1972. Since 1977 my research has progressively employed a range of recombinant techniques to isolate, clone and express genomic DNA and cDNAs encoding various proteins from and in a variety of different organisms including prokaryotes (bacteria), simple eukaryotes like yeast, and animal cells. Over this period I have observed either first hand or from a close distance the research that others in my field were conducting.

1.10 Since returning to Australia in 1981, I have taken a very active role in the conduct and development of molecular biology in Australia. I am a member of the Board of two other national facilities concerned with advanced molecular biology, the Australian National Genome Information Service (ANGIS) and the Australian Proteome Analysis Facility (APAF), and of several Advisory Boards of major institutions and companies active in basic and applied research in molecular biology. Details of the committees and Boards that I have been involved with over the last twenty years are provided in my curriculum vitae (JSM-1).

1.11 I have advised the Queensland State Government and the Prime Minister's Science, Engineering and Innovation Council on issues in genome science, gene technology and biotechnology, and am a member of the Queensland Bioindustries Advisory Council and a member of the Executive of BIOCOG, (Biotechnology Consultative Group) which advises the Federal Minister for Industry, Science and Resources on the

development of the biotechnology industry in Australia. I have also represented Australian molecular biology and biotechnology at senior meetings in the United States, Europe and Japan, and was involved recently in the agreement between Celera Genomics Corporation and the NHMRC, to make available Celera's human, mouse and Drosophila genome databases available to Australian biomedical researchers.

1.12 I have spent research periods in Sydney, Melbourne, Brisbane, Houston, Cambridge and Oxford, and am familiar with most molecular biological research groups in Australia. I have visited many of the major international institutions involved in molecular biology and genome research. I am a frequent guest speaker at national and international conferences in relation both to my own work and to the development of molecular biology in general. For example, I delivered the keynote addresses at the 1995 Conference of the Genetics Society of Australia and the 1996 Conference of the Australian Society for Medical Research, and an invited plenary address at the 2000 International Congress of Endocrinology. I believe this contact with the field has allowed me to become familiar with the field nationally and internationally, and with the general level of skill possessed by those working in this field in Australia.

1.13 Further, as a research scientist and the leader of an active research group, the Professor of Molecular Biology at the University of Queensland, the Director of the ARC Special Research Centre for Molecular and Cellular Biology, the Director of the ARC Special Research Centre for Functional and Applied Genomics, the Director of the Australian Genome Research Facility, and the Co-Director of the Institute for Molecular Bioscience, I have been (and am) required to have and to maintain a strong working knowledge of the Australian and international scientific literature concerning molecular biology. I have learned about molecular biology through my research training and activities, postdoctoral experience in the United States, sabbatical experience at the University of Cambridge and the University of Oxford, publications, research supervision of postdoctoral fellows and Ph.D., honours and undergraduate students, my professional involvement with numerous Australian and International organisations and industries, attendance at scientific conferences and symposia, and referee duties for various local and international journals and organisations. When I visit other research groups I often discuss with them recent developments in my field of

expertise. I would also convey relevant knowledge gained from those discussions to my research team upon my return.

2 MY INSTRUCTIONS

2.1 Human Genome Sciences Inc's (HGS') Patent Attorneys have provided me with a copy of a document entitled "*Guidelines for Expert Witnesses in Proceedings in the Federal Court of Australia.*"

2.2 HGS' Patent Attorneys have also provided me with copies of the following documents:

2.2.1 The patent specification accompanying Australian Patent Application 696764 (AU-B-73941/94) in the name of Human Genome Sciences Inc ("HGS"), entitled "Vascular Endothelial Growth Factor 2" ("the patent specification"); and

2.2.2 The Statutory Declaration of Peter Adrian Walton Rogers that was executed on 16 February 2000 ("Associate Professor Rogers' statutory declaration").

2.3 I have been asked to do the following with each of the documents identified in paragraph 2.2:

2.3.1 To review the patent specification and to describe what it would have conveyed to me had I read it in March 1994;

2.3.2 To review and provide comments on Associate Professor Rogers' statutory declaration; and

2.3.3 To base all comments presented herein on my knowledge as at 8 March 1994, unless I specifically state otherwise.

I have done this and my comments are set out in this Statutory Declaration.

2.4 Now exhibited to me and marked with the letters "**JSM-3**" is list of the documents provided to me by HGS' Patent Attorneys.

2.5 In this statutory declaration I refer to and discuss a number of documents. I am informed by the Patent Attorneys representing HGS in these proceedings that a copy of each of the documents that I discuss in this declaration will be separately filed and served in support of this opposition.

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3 THE PATENT SPECIFICATION

3.1 I am informed by the Patent Attorneys for HGS that the HGS Patent Specification AU-B-696764 has an earliest date of filing of March 8, 1994 ("the HGS priority date"), which was established by the filing of United States patent application 08/465,968 ("the priority application"). I am also informed by HGS' Patent Attorneys that subsequently a PCT application was filed on 12 May 1994 [12.05.94] ("the HGS filing date"), which claimed the benefits of the priority application. I am further informed by the patent attorneys for HGS that the PCT application was published on 25 September 1995 [25.09.95] in Australia and on 14 September 1995 [14.09.95] by the World Intellectual Property Organisation (WIPO), entered the national phase in Australia and was published as accepted on 17 September 1998 [17.09.98].

3.2 The patent specification relates to VEGF-2 polynucleotide sequences; polypeptides that are encoded by those polynucleotide sequences; uses of the polynucleotide and polypeptide sequences; means for isolating the polynucleotide sequences; and means for producing the encoded polypeptide sequence. As of March 1994 I was generally familiar with what was known and practised in the field of molecular biology in Australia and overseas and, in particular, the use of molecular biological techniques in isolating, cloning and expressing protein, although I was not specifically working with the PDGF/VEGF gene family.

3.3 HGS' Patent Attorneys have informed me that VEGF-2 and VEGF-C are the same molecule. The fact that VEGF-2 is now known by another name does not cause me any difficulty in understanding the information in the patent specification. When a new protein is identified it is usually named by the first group or groups to discover it, unless there is a precedent concerning the naming of the new protein because of its relationship to others (i.e. it belongs to a family members which has been described previously).

3.4 Before discussing the information provided in the patent specification I have been asked to comment on (a) the environment in which molecular biologists operated in 1994 in Australia, and (b) the field of gene cloning and protein expression as at 1994.

(a) The Field of Molecular Biology in Australia in 1994.

3.5 By 1994 many laboratories in Australia were using molecular biological techniques. These included virtually every department of biochemistry, microbiology and genetics in the different universities around Australia, many research organisations and institutes such as the CSIRO, the Walter and Eliza Hall Institute of Medical Research, the Queensland Institute of Medical Research, the Garvan Institute of Medical Research (among many others) and companies such as Biotech Australia Pty. Ltd. and Commonwealth Serum Laboratories Ltd. I was generally aware of this by reason of my own work and experience. I routinely met with other scientists from other laboratories and institutes during visits to those places and during conferences during which we would discuss both research findings and methodologies. I also supervised and maintained contact with graduate and postdoctoral students whom I supervised and worked with or who had been in the laboratories in which I have worked. Information and knowledge gained from those discussions would be conveyed by me to my research team.

3.6 The normal working group in a laboratory using molecular biological techniques would consist of an experienced Ph.D. graduate (a research scientist or academic staff member) advising more junior Ph.D. graduates (postdoctoral fellows or research officers) and/or research assistants and/or graduates and/or Ph.D. students. In my experience, a person of ordinary skill in my field of technology was and is someone who has the necessary skills to perform a particular task, rather than a person simply with a certain level of academic qualification. Moreover such a person is someone who is capable of designing and carrying out experiments with a minimum degree of supervision. People with a basic degree, working as research assistants, would often be taught in a short period of time how to use molecular biological techniques in a professional manner and would thereafter become the researcher(s) who conducted the research at the laboratory bench. Sometimes that work would be under the strict direction of a person with a Ph.D and other times it would be at their own initiative, subject to general direction, depending on the abilities of that researcher.

3.7 Generally, one of the avenues by which I and other scientists in Australia keep up-to-date with developments in our field is through reading research (journal) articles. There are a number of journals that are, and

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were before 1994, commonly read by most biological scientists, some of those publications included *Nature*, *Science*, *Proceedings of the National Academy of Science (USA)*, and *Cell*. After that a researcher would tend to specialise in what they read, taking the best papers from journals that are specific for their area of interest.

3.8 Scientists in Australia, as do scientists elsewhere, also constantly observe what is happening overseas through international conferences and international contacts. Before 1994 and now, there were (are) a lot of international conferences which Australian scientists frequently attended.

3.9 In the field of molecular biology, in the mid-1990's and today, the background knowledge of researchers in Australia was the same as or similar to that of a researcher in the USA, UK, Europe, Japan and elsewhere. It could be that in the laboratories in the USA people might have heard things on the grapevine before they were published, which might not have been the situation quite so often in Australia. But apart from that, there is and was very little difference - it is and was very much an international community of scientists.

3.10 Before March 1994 (and today) many Australian scientists who did a Ph.D. in the field of molecular biology would go overseas to do post-doctoral studies. The great value in this is that these scientists get to know personally a set of people that are about the same age and at about the same stage of development, as well as more senior people. They usually keep in touch with such people throughout their professional life, and so set up information networks that develop with the years. Furthermore, such interactions as well as those at international conferences regularly lead to reciprocal visits to other laboratories.

3.11 There is little difference in the level of skill between Australian researchers in the field of molecular biology and researchers in other industrialised countries around the world. A lot of world-class work has been done in Australia and that is reflected in many publications that have come from Australia. For example, work carried out in my research centres, some of which I was directly involved with, has been published in *Nature Genetics*, *Cell*, and the *Proceedings of the National Academy of Science (USA)* - which are regarded as three of the top journals in the field. The amount of work to have come out of Australia is quantitatively

lower than that from other countries such as the United States and United Kingdom, because the number of people involved is lower, but the per capita publication rate and overall quality of the work carried out in Australia over the past three decades has been similar to that of other advanced countries, and in my opinion has been, for the most part, first rate. I believe that the work of our group is just one example that shows that Australian scientists in the field of molecular biology were often and still are the leaders in the field.

(b) Gene cloning and protein expression

3.12 By 1994 the field of gene isolation, cloning and protein expression in Australia was comparable to that in any other industrialised nation. Rapid advances in molecular engineering techniques and in particular gene amplification techniques provided researchers with a versatile set of techniques and tools for isolating and amplifying genomic sequences.

3.13 An important difficulty that researchers faced in 1994 (and still face today) is the process of determining what a new gene encodes. This involves careful consideration and scientific training, and it is not a simple or straightforward process. Importantly, the isolation of a DNA sequence does not guarantee sufficient information to establish whether the sequence encodes a protein or if it does, the nature and function of the protein it might encode. Such information had to be determined in 1994 (as it is today) by a researcher using scientific skill, their experience, their knowledge and often a wide range of different analytical and experimental tools.

3.14 However, once a DNA sequence had been cloned, further manipulations of that sequence would be relatively routine practice. Moreover once a protein sequence had been identified there were many routine methods available for analysing that protein. Computational analysis provided one method, however there were other methods, which relied on more direct experimental analysis. Researchers often used a combination of these methods to characterise a newly discovered protein. For example, by 1994 researchers were well acquainted with the fact that hydrophobic/hydrophilic characterisation and/or identification of conserved features of an amino acid sequence can indicate likely structural or functional characteristics. However, they were also well aware that such analytical tools had their limits.

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3.15 Where a researcher thought an identified protein might contain a secretion signal sequence they often searched the sequence for amino acid motifs characteristic of such a sequence. Computer analysis programs available in 1994 for examining proteins for secretion signal or leader sequences included P SORT and SIGNAL P.

3.16 Once the gene sequence encoding a protein had been identified, researchers could apply well-established molecular biology techniques to produce the protein in any desirable form. Expression of a known gene was a relatively routine and standard laboratory activity by 1994, *albeit* one that required care and attention. Expression of a known gene sequence involved: (a) selection (*a priori* or by trial and error) of an appropriate host-vector expression system that produced the protein in a form that would meet the end user's requirements; (b) insertion of the gene sequence into the selected vector(s); (c) introduction of the vector(s) into an appropriate host cell(s); (d) cultivation of the host cell; and (e) recovery of the expressed protein.

3.17 By 1994, when researchers were selecting expression strategies they would typically take into consideration such factors as their experience, the type of protein to be expressed, morphological and physical features of the protein (e.g. whether the protein contained a signal or leader sequence), the physiological activity of the protein and the use to which that protein was to be put.

3.18 In conducting expression experiments it was not uncommon or unusual (nor is it uncommon or unusual today) for researchers to encounter obstacles. Many such obstacles can and could be overcome by the application of ordinary skill. An example would be where the researcher was working on expression of a protein and found that the protein was not released from the cell. There are a number of reasons why this problem might have arisen and I would expect that a person of ordinary skill in molecular biology would be aware of those reasons. An obvious possibility would be that the signal sequence, necessary for the secretion of the protein from the cell, was missing or faulty. This could be remedied by adding a heterologous signal sequence as is and was routinely done. Researchers often accommodated such situations by considering and trialing a series of different expression strategies and systems. In this respect, my team and I usually assess a bank of

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different expression strategies and systems simultaneously, or at least in close sequence, when expressing a protein for the first time.

3.19 There were in 1994 many expression vectors that were commercially available or could be obtained from the author of the publication in which they were reported. Biotechnology company catalogues describing expression vectors and associated host cells in which they could be used were part of every researcher's standard operating texts and invariably contained all the essential information that a researcher required to express a particular protein. These catalogues regularly contained additional information about the system including a reference to one or more seminal papers that described the expression vector, examples of other papers where the vectors had been successfully used and information about how to purchase the vectors and their hosts.

(c) The patent specification

3.20 The patent specification relates generally to VEGF-2 polynucleotide and polypeptide sequences and provides such sequences for diagnostic, therapeutic and prophylactic use.

3.21 On page 3 (lines 30 to 32) the patent specification states:

> "In accordance with one aspect of the present invention, there is provided a novel mature polypeptide which is a VEGF-2 as well as fragments, analogues and derivatives thereof."

3.22 The information provided in Figure 1 in the patent specification discloses a VEGF-2 sequence that is 350 amino acid residues in length. For the reasons mentioned in paragraph 4.7 below, the nucleotide sequence disclosed in Figure 1 may also be read to encode an additional 23 amino acids.

3.23 After identifying the invention so far as it relates to VEGF-2 polypeptides the patent specification states on page 4, lines 1 to 3:

> "In accordance with another aspect of the present invention, there is provided polynucleotides (DNA and RNA) which encode such polypeptides."

3.24 The information provided in Figure 1 in the patent specification discloses a VEGF-2 polynucleotide sequence that is 1525 base pairs in length.

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3.25 After identifying VEGF-2 polypeptides and polynucleotides, the patent specification describes uses of those sequences. The patent specification states that the sequences can be used for therapeutic purposes such as would healing, to promote growth of damaged bone and tissue, to promote endothelialization of damaged tissues as well as diagnostics for tumours, cancer therapy, to produce antibodies against VEGF-2 and to produce or identify antagonists and inhibitors that may be used to inhibit the action of VEGF-2 to, for example, prevent tumour angiogenesis or prevent tumour neovascularisation.

3.26 On page 5 (lines 13 to 18), the patent specification refers to a deposited sequence i.e. ATCC Accession No. 75698 (plasmid 182;618) on 4 March 1994. I note that the specific details that identify the deposited clone have been written into the patent specification by hand. I am informed by the Patent Attorneys representing HGS that appropriate procedures were followed to introduce this information into the patent specification and that I am to consider the hand written information as part of the specification. I am proceeding on that understanding.

3.27 After identifying the deposit sequence the patent specification provides information about the tissue source from which the identified VEGF-2 coding sequences could be isolated. This information provides a source from which a VEGF-2 clone could be isolated and expressed. The patent specification also provides additional information about alternate tissue sources in Figure 5. Figure 5 depicts the results of northern blot analysis of VEGF-2 in a range of human adult tissues. The information contained in Figure 5 is discussed in more detail below.

3.28 On page 5 lines 24 and 25 of the patent specification, HGS identify their VEGF-2 protein as being structurally related to the PDGF/VEGF family. When comparing their protein against other PDGF/VEGF family members they observed that the VEGF-2 sequence that they identified contains a fourteen amino acid signature motif that is common to the PDGF/VEGF family of growth factors, as well as eight cysteine residues that are conserved amongst VEGF, PDGFα and PDGFβ family members. Further, they also observed that VEGF-2 shares some sequence identity with each of these family members, that identity being VEGF (30%), PDGFα (23%) and PDGFβ (22%). Figure 3 presents a comparison of the percentage homology between VEGF-2, VEGF, PDGFα and PDGFβ.

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3.29 After providing a brief characterisation of VEGF-2, HGS provides information concerning production of a recombinant product with one or more of the biological properties of VEGF-2. For example, they describe methods for constructing a large range of expression vectors and expression systems suitable for use in a range of prokaryotic and eukaryotic host cells (see e.g. pages 10 to 15 of the patent specification). They describe host cells for expression, including mammalian cells, yeast cells, insect cells, and procaryotic cells (see e.g. pages 10 to 15 of the patent specification) and the use of heterologous signal sequences to achieve efficient secretion (see e.g., page 14, lines 6-23). By March 1994 all of these techniques were routinely available from standard texts, and the indicated combinations and variations were commonly employed by those skilled in the art.

3.30 The patent specification then describes examples of diagnostic and therapeutic uses to which VEGF-2 products may be put.

3.31 Therefore, in summary, in my opinion the patent specification provides, *inter alia*, the following:

3.31.1 DNA sequences encoding VEGF-2 amino acid sequences and fragments thereof;

3.31.2 Cell sources for the identification and isolation of VEGF-2 coding sequences;

3.31.3 Methods for producing recombinant VEGF-2 in eukaryotic and prokaryotic host cells;

3.31.4 Methods for producing abundant amounts of a polypeptide isolated from recombinant cells having the *in vivo* activity of VEGF-2;

3.31.5 Methods for producing antibodies against VEGF-2 polypeptides for the purification of VEGF-2;

3.31.6 Uses of VEGF-2 for stimulating angiogenesis;

3.31.7 Uses of VEGF-2 polypeptides for stimulating wound healing and for vascular tissue repair;

3.31.8 Antagonists to inhibit VEGF-2 activity and anti-sense constructs to inhibit VEGF-2 expression;

3.31.9 Agonists against VEGF-2 polypeptides;

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3.31.10 Diagnostic methods for identifying mutations in the VEGF-2 coding sequence and alterations in the concentration of VEGF-2 protein in a sample derived from a host.

3.31.11 A method to test for *in vitro* biological function or activity of VEGF-2;

3.31.12 Pharmaceutical carriers and delivery systems for the VEGF-2 polypeptide as well as means for gene therapy to provide therapeutic and prophylactic effects against a wide range of different disease states;

3.31.13 Uses of the VEGF-2 polypeptide and gene sequence for the treatment of disease related medical conditions such as myocardial infarction (heart attacks) and ischemia; and

3.31.14 Uses of the VEGF-2 polypeptide and gene sequence for the treatment of non-disease related medical conditions such as burns, injuries and plastic surgery.

3.32 A more detailed but non-exhaustive list of the information that is disclosed in the HGS patent specification is provided in Table 1. Now exhibited to me and marked with the letters "**JSM-4**" is Table 1.

3.33 In my opinion, the disclosed VEGF-2 DNA and amino acid sequence information, the disclosed cell sources for isolating additional VEGF-2 clones, and the description for cloning and expressing VEGF-2 provide all of the information that I would have needed to express a biologically active VEGF-2 using routine scientific application. All of this information is provided in the patent specification. Indeed, without this information not only would I not have been able to identify and isolate VEGF-2 and VEGF-2 coding sequences in March 1994, I would not have known that the protein existed.

3.34 Further, the identified sequence information provided in the patent specification would have allowed me and I believe any person of ordinary skill in the field of molecular biology in 1994 to design specific strategies to obtain any polynucleotide sequence (ie gDNA, mRNA or cDNA) encoding VEGF-2. For example, the sequence information could have been used to design highly specific DNA primers that exactly match the VEGF-2 sequence, thus enabling the isolation of gDNA or cDNA VEGF-2 clones from suitable libraries or from the tissue sources identified in the patent specification.

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4 Associate Professor Rogers' Statutory Declaration

4.1 I have been asked to read and comment on the Statutory Declaration by Associate Professor Rogers. I have done this and make the following comments concerning that declaration.

General comments

4.2 Associate Professor Rogers' statutory declaration raises a number of general issues concerning the patent specification. In the following passages I will address those issues and then turn my attention to some of the specific comments that he makes.

(a) Expression of the VEGF-2 sequence

4.3 The first issue that Associate Professor Rogers puts forward is that expression of the VEGF-2 sequence would have been problematic based on the information in the patent specification because the full length VEGF-2 sequence is not disclosed in the specification and the patent specification suggests that the first 24 amino acids of the disclosed sequence may represent a secretion signal sequence. I do not agree with his perspective. I believe that a person of ordinary skill in the field of molecular biology would have been able to deal with these issues easily by applying routine trial and experimentation to produce the VEGF-2 protein, had they been presented with the information in the patent specification.

4.4 It would have been a reasonable proposition in 1994 to suggest that the first 24 amino acids might be an atypical signal sequence. I note that Dr Alitalio and his research team appeared to draw a similar conclusion in 1996 in their publication reporting VEGF-C (see: Joukov *et al.*, (1996), *EMBO Journal* 15: 290-298).

4.5 Had I attempted to express the VEGF-2 protein using the putative secretion signal sequence identified in the patent specification and had that not worked, I and I believe any other person of ordinary skill in the field of molecular biology would have considered two obvious solutions to produce VEGF-2.

4.6 First, I would have gone back to look more closely at the putative signal sequence and would have noted that it deviates in some respects from typical signal sequences. To overcome this I would have used a

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heterologous signal sequence as is and was routine in the field of molecular biology in 1994, and which is taught in the patent specification on page 7 (lines 21 to 34), page 8 (lines 1 to 4), pages 9 to 10 (lines 34 to 5), and page 14 (lines 17 to 23).

4.7 Second, I would have considered whether the signal sequence was incorrect or incomplete. In considering this issue I would have gone back and read the specification more carefully and would have noted that the specification is not entirely clear about the existence of the signal sequence. In particular, I would have observed that the specification states on page 5 (lines 25 to 28), that the first 24 amino acids are only likely to represent a signal sequence. I would have also noted that the sequence in Figure 1 possibly encoded a further 23 continuous amino acids upstream of the suggested methionine start codon. Thus, I would have looked in an appropriate cDNA library for larger VEGF-2 clones that might encode more sequence information and a *bona fide* signal sequence. In this respect I note that in Figure 5, a 2.2 kb mRNA species was detected, which I would expect to encode the full length sequence of VEGF-2 (the full length VEGF-2 sequence is now known to be transcribed as a 2.1 kb mRNA species). Any experimentation required to identify potential upstream sequences from the VEGF-2 mRNA species would be routine.

4.8 Taking into account the existence of the additional 23 amino acids at the N-terminal end of the VEGF-2 sequence disclosed in the patent specification, I would also have stitched a signal secretion sequence to the beginning of the cDNA disclosed in the patent specification, as is taught in the patent specification. I note that such an experiment was done and is described in Australian Patent Application 60467/96 (714,484) (HGS' second VEGF-2 patent application) and the resultant product from those experiments is reported to be biologically active (see page 42, line 32 to page 43 line 25 and Figures 8 and 9 -- HGS' second VEGF-2 patent application).

4.9 The patent specification describes the use of such heterologous signal sequences on page 7 (lines 21 to 34), page 8 (lines 1 to 4), pages 9 to 10 (lines 34 to 5), and page 14 (lines 17 to 23). On these pages reference is made to combining the mature sequence with a leader (secretion) sequence. Using such a system I would have been confident that I could

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have produced a secreted form of the VEGF-2 amino acid sequence disclosed in the patent specification.

4.10 In addition to the above experiments I might also have attempted to express the 350 amino acid form of the protein, and or other fragments of VEGF-2 as taught in the patent specification. Such experiments could all have been performed in parallel with routine ease. By 1994 there were a large range of methods available to researchers for doing this type of work. Furthermore, systems for carrying out such research were well established and routinely available.

4.11 I believe either of these two approaches would have produced the desired outcome of producing VEGF-2, which I note has subsequently been shown to be the case.

4.12 I have been asked to consider and comment on US patent 6,130,071 entitled "Vascular Endothelial Growth Factor C (VEGFC) ΔCYS_{156} Protein and gene, and uses thereof". This patent describes, amongst other things, the expression of VEGF-C fragments using methodologies consistent with the strategy proposed above and which were routinely available and followed in protein expression laboratories prior to 1994. In particular, a heterologous secretion signal sequence was used to express and produce biologically functional or active fragments of VEGF-C. Accordingly I believe the same principles and strategy would apply and that the results in that patent reinforce that VEGF-2 sequences may be produced using the strategy that discussed to produce a biological function or activity VEGF-2 protein.

4.13 Therefore in my opinion, the fact that the signal sequence information was incomplete would not have presented me with a formidable or even significant problem, given that the patent specification provides that a heterologous signal sequence may be used to achieve efficient secretion, and it would have involved routine experimentation to do so. I believe that by applying the information in the patent specification and a reasonable amount of routine trial and experimentation using standard protein expression and secretion techniques I could have easily produced a secreted VEGF-2 protein.

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(b) **Biological assay**

4.14 Associate Professor Rogers asserts that the patent specification fails to provide an assay to test for VEGF-2 biological function or activity.

4.15 I note that on page 18 (lines 6 to 8) HGS refer to a use of VEGF-2 for the *in vitro* expansion of vascular endothelial cells. I would understand from reading that passage that an expected activity of VEGF-2 is to promote growth of vascular endothelial cells in culture. I could use such an activity to verify the production of VEGF-2 as taught by the patent specification and I note that such an activity has subsequently been shown to be something possessed by VEGF-2. I refer to HGS Patent Application 60467/96 (714,484), which shows that VEGF-2 exhibits proliferative effects on vascular endothelial cells (see page 42, line 32 to page 43 line 25 and Figures 8 and 9).

4.16 In addition, had I wanted to examine a secreted VEGF-2 protein using other assays relevant for activities specified in the patent specification (see below) I would have identified someone working in the vascular biology or endothelial biology field and I would have asked them for advice about the types of angiogenic assays that were available in 1994 and how to set up the assays, or I would have asked whether they would be prepared to collaborate with me in my research by testing the protein that I had produced. By 1994 it was common and routine in Australia for researchers to collaborate on projects. Alternatively, I could have done a basic Medline search for assays that tested for angiogenic activity since the patent specification tells me that VEGF-2 is an angiogenic molecule (see page 1, 4 and 16 to 18 of the patent specification). I would then have read those papers and I would have set up appropriate assays. Among the publications that I would have located here are some examples:

4.16.1 Passaniti A, et al. (1992) "A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor." *Lab. Invest.* 67:519-528

4.16.2 Splawinski J, Michna M, Palczak R, Konturek S, Splawinska B (1988) "Angiogenesis: quantitative

assessment by the chick chorioallantoic membrane assay." *Methods Find. Exp. Clin. Pharmacol.* 10:221-226

4.17 Therefore, I disagree that the patent specification does not provide sufficient information to undertake a biological assay for VEGF-2.

(b) Biological activity

4.18 Associate Professor Rogers asserts that the patent specification fails to demonstrate a biological activity of VEGF-2 (see, for example, paragraph 4.6.2 in Associate Professor Rogers Statutory Declaration). The patent specification provides extensive guidance for the *in vitro* and *in vivo* biological activities and uses for VEGF-2. Further the patent specification provides in Example 1 Northern Blot data (see figure 4) showing that VEGF-2 is over-expressed in breast cancer cell lines. This result indicates to me that VEGF-2 is biologically active in tumours.

4.19 According to the patent specification, VEGF-2 is a molecule that is capable of re-vascularizing damaged tissue and/or has angiogenic properties (see page 16 lines 27 to 29 of the patent specification).

4.20 On page 4 of the patent specification, HGS state that the present invention concerns a protein that possesses angiogenic behaviour (see, for example, page 4 lines 7 to 14 and lines 18 to 21).

4.21 On pages 16 to 24, the patent specification discloses a multitude of biological applications for the protein. In particular, it discloses:

(a) *in vivo* uses of the proteins such as in wound healing or re-vascularizing damaged tissue (see page 16 line 27 to page 17 line 28);

(b) *in vitro* uses of the protein, such as for the generation of inhibitors of angiogenesis and neovascularization (see page 17 line 29 to page 18 line 5) and for *in vitro* culturing of vascular endothelial cells (see page 18 lines 6 to 8); and

(c) immunological uses of the protein, as diagnostics to detect the presence of tumours in certain individuals (see page 24 lines 5 to 9) or as antagonists to treat tumours or inflammation caused by increased vascular permeability (see page 24, lines 2 to 4).

4.22 The patent specification is not restricted to the above uses of VEGF-2 proteins. It also discloses uses of VEGF-2 polynucleotides as well as uses of truncated VEGF-2 proteins. Uses of the polynucleotide sequences disclosed in the patent specification include:

4.22.1 As probes and or primers (see page 8 and Example 2).

4.22.2 In gene therapy -- Including both *ex vivo* and *in vivo* applications (see page 18 (lines 9 to 34)).

4.22.3 To identify the chromosomal location of the genomic sequence encoding VEGF-2 (see page 20 and 21).

4.22.4 For the determination of genetic diseases associated with VEGF-2 (see page 20 and 21).

4.22.5 For *in vivo* inhibition of VEGF-2 by the use of antisense technology.

4.23 On page 24 (lines 25 to 31) the patent specification identifies uses of truncated versions of VEGF-2 for inactivating the activity of endogenous VEGF-2. It also discloses how such truncated molecules may be used therapeutically as anti-cancer drugs, to prevent inflammation or to treat solid tumour growth, diabetic retinopathy, psoriasis and rheumatoid arthritis (page 25 lines 4 to 13).

4.24 Therefore, in my opinion the patent specification discloses a comprehensive range of uses of both the polynucleotide and polypeptide sequences of the invention. Such information constitutes the basic information that I would have required in 1994 to use VEGF-2 in a wide range of biological activities.

(d) The claims in the patent specification do not encompass VEGF, PDGF and PlGF

4.25 I note that Associate Professor Rogers suggests that the claims in the patent specification could *in extremis* cover molecules like VEGF, PDGFα, PDGFβ and PlGF because they include the words "fragments, analogues and/or derivatives". I do not agree with his position. In my opinion Associate Professor Rogers' comments concerning this issue are spurious and would not reflect the reasonable view of anyone experienced in the field of molecular biology.

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4.26 I would expect VEGF-2 fragments, analogues and derivatives to have greater structural similarity to VEGF-2 than other related proteins such as VEGF and PDGF, which exist as distinct proteins in nature.

4.27 Having regard to the information provided in the patent specification I note that HGS clearly distinguishes VEGF-2 from VEGF, PDGFα and PDGFβ. Figure 2 provides a comparison of the amino acid sequence of VEGF-2 and other members of the PDGF/VEGF family, which demonstrates the low level of homology between these proteins. Figure 3 presents a comparison of the percentage homology between these molecules. Using this information I could and would distinguish VEGF-2 fragments, analogues and/or derivatives from VEGF, PDGFα or PDGFβ.

(e) Hybridisation with VEGF-2

4.28 Associate Professor Rogers asserts in his Statutory Declaration that:

> "...the claim limitations directed to polynucleotide hybridization do not serve to distinguish the prior art that discloses VEGF, PlGF, or PDGF polynucleotides and/or polypeptides, because any DNA can hybridize to VEGF-2 DNA if the hybridization conditions (*e.g.*, temperature and ionic strength) are sufficiently relaxed. Neither the claims nor the specification require a level of hybridization stringency that would exclude VEGF, PlGF, or PDGF polynucleotides or encoded polypeptides from the scope of the claims. (No minimum level of hybridization stringency is required at all.)"

4.29 The claims in the patent specification refer to hybridization with a VEGF-2 polynucleotide sequence. When I read such language in combination with the description of hybridization set forth in the patent specification I would understand it to mean that hybridization conditions should be sufficiently specific to exclude known non-VEGF-2 sequences.

4.30 The patent specification provides examples (see Example 1) of hybridization conditions that could be used to specifically identify VEGF-2 sequences. Further the patent specification states that there is no detectable homology at the nucleotide level between VEGF-2 and VEGF and PDGF. By 1994 it would have been routine for me and I believe any other researcher in the field to use the hybridization conditions put forth in the specification such that there would be no cross hybridization with VEGF, PlGF, or PDGF. In this respect I refer to paragraph 2.4.3 in Associate Professor Rogers' Statutory Declaration where he indicates

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that scientists could, prior to 1994, exercise considerable control over the stringency of hybridization conditions. I would agree with this statement.

(f) VEGF 2 antibodies

4.31 Between pages 22 and 24 of the patent specification there is a description of methods for making antibodies to VEGF-2. Those methods were well known in the scientific literature and easy to perform in 1994.

4.32 In Sections 2 and 3 of Associate Professor Rogers' Statutory Declaration he repeatedly asserts that antibodies that bind to conserved regions of VEGF-2 would cross-react, to some extent, with the similar regions in VEGF or PDGF or PIGF. For example, in paragraph 2.5 he states:

> "From the theoretical universe of all possible antibodies that bind to VEGF-2, a molecular biologist with common general knowledge would reasonably expect that some antibodies which bind to these conserved regions (epitopes) of VEGF-2 would cross-react, to some extent, with the similar regions in VEGF or PDGF or PIGF. In other words, VEGF, PDGF, and PIGF are polypeptides which bind an antibody that binds (or is capable of binding) to VEGF-2. The expected antibody cross-reactivity means that the claim limitation relating to antibody binding does not exclude prior art VEGF or PDGF or PIGF polynucleotides and polypeptides from the scope of claims."

4.33 Associate Professor Rogers' comments are, in my opinion, extremely speculative. The fact that there is some sequence homology between VEGF-2 and VEGF and other proteins does not mean that there is a high probability that antibodies to VEGF-2 will cross react with VEGF or those other proteins. Antibodies generally have exquisite specificity and will only cross react with closely related proteins. I do not believe that any conclusion can be drawn about cross-reactive antibodies at a theoretical or practical level.

4.34 Further, I note that Associate Professor Rogers asserts that claim 49 includes within its scope antibodies raised against VEGF, PDGF or PIGF polypeptides. In particular he states: "... such antibodies are explicitly disclosed or suggested in...". None of the prior art documents cited by Associate Professor Rogers establish that the described antibodies bind VEGF-2. In my opinion, no conclusion can be reached about the cross-reactivity of these antibodies based on the disclosures in these publications.

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Specific Comments

4.35 In this section I address some of the more specific comments that Associate Professor Rogers makes in his statutory declaration. My decision not to address each and every paragraph in that declaration should not be taken as acceptance of the paragraphs to which I do not refer.

Paragraph 2.2.2

4.36 In paragraph 2.2.2 Associate Professor Rogers states:

> "A substantial number of amino acid changes are required to change the VEGF-2 sequence into, *e.g.* the VEGF or PDGF sequences shown in Fig. 2A-2B. However, I find no limitation in the specification or the claims setting a maximum number of modifications that may be performed to generate a "fragment, analogue, or derivative" within the scope of the claim. Thus, polypeptide and polynucleotide claims which recite "fragment," or "analogue," or "derivative" effectively encompass prior art polypeptides and polynucleotides, including but not limited to VEGF, PlGF, and PDGFα and PDGFβ polypeptides and polynucleotides."

4.37 I would not require the patent specification to set a maximum limit of modifications that can be made to a protein before I could reasonably ascertain whether a protein was a fragment, analogue and/or derivative of VEGF-2. In this respect I refer to my comments in paragraph 4.25 to 4.27. As soon as a protein starts to look more like VEGF, PDGFα, PDGFβ, or PlGF, it would not, in my opinion, be a fragment, analogue and/or derivative of VEGF-2. Thus, I would not regard any of VEGF, PlGF, PDGFα or PDGFβ to be VEGF-2 fragments, analogues and/or derivatives.

Paragraph 2.4

4.38 In paragraph 2.4 Associate Professor Rogers states:

> "Some of the claims directed to polynucleotides or polypeptides include a limitation relating to "hybridizing" to (the complement of) a VEGF-2 polynucleotide or portion of a VEGF-2 polynucleotide. (See, *e.g.* claims 16-20, 34-45, and other claims dependent therefrom.) However, the claim limitations directed to polynucleotide hybridization do not serve to distinguish the prior art that discloses VEGF, PlGF, or PDGF polynucleotides and/or polypeptides, because any DNA can hybridize to VEGF-2 DNA if the hybridization conditions (*e.g.* temperature and ionic strength) are sufficiently relaxed. Neither the claims nor the specification

require a level of hybridization stringency that would exclude VEGF, PIGF, or PDGF polynucleotides or encoded polypeptides from the scope of the claims. (No minimum level of hybridization stringency is required at all.)"

4.39 As I read the claims I do not read them as being directed to VEGF, PIGF, or PDGF. I believe the patent specification provides sufficient information and direction to a skilled reader to understand that the hybridization language used in the specification and recited in the claims do not include polynucleotides encoding VEGF, PIGF or PDGF. Given that there is no detectable homology at the nucleotide level between VEGF-2 and the other members of the PDGF/VEGF family, these sequences will not cross hybridize, particularly using the hybridization conditions recited in the claims. I refer to and repeat my comments in paragraphs 4.28 to 4.30 above, which addresses this matter in more detail.

Paragraph 2.7

4.40 In paragraph 2.7 Associate Professor Rogers refers to a number of publications, which he says teach the subject matter claimed in the patent specification. I have reviewed each of documents **D1, D5, D7, D12, D16, D18, D19, D20, D29, D34, D35, D36, D39** and **D41**. None of these documents describe VEGF-2. Further, to the extent that one or more of the claims in the patent specification might include a polypeptide that binds an antibody that binds to VEGF-2 I note that none of these publications describe an antibody that binds to VEGF-2 or establishes cross-reactivity between VEGF-2 antibodies and VEGF antibodies. These documents refer to such subject matter as VEGF, PDGF and PIGF. In my opinion they are not relevant to the subject matter claimed in the patent specification for the reasons mentioned in paragraphs 4.31 to 4.34, above.

Paragraph 2.7.2 (footnote)

4.41 In the footnote to paragraph 2.7.2 Associate Professor Rogers states:

"...the opposed application only exemplifies an isolated cDNA, not an isolated VEGF-2 RNA or VEGF-2 genomic DNA."

4.42 However, at paragraph 3.4.3 Associate Professor Rogers states:

"To the extent that the prior art had not explicitly isolated RNA or genomic DNA encoding any of these three polypeptides, it is my opinion that such RNA and genomic DNA was no more than routine variation over prior art disclosures of cDNAs..."

4.43 The patent specification clearly identifies an isolated mRNA encoding VEGF-2. For example, Example 1 and Figures 4 and 5 of the patent specification teaches the isolation and detection of an mRNA species (see also the patent specification at page 27, lines 4 to 32). Furthermore, the isolation of VEGF-2 genomic DNA would have been a routine and straightforward task in 1994 for any person of ordinary skill in the field of molecular biology given the information in the patent specification.

4.44 Hence I would agree with Associate Professor Rogers' comments to the extent that they can be interpreted to mean that RNA and genomic DNA was a routine variation over the sequence information provided in the patent specification.

Paragraph 2.7.4

4.45 In paragraph 2.7.4 of Associate Professor Rogers' statutory declaration reference is made to a Response filed by HGS to the Australian Patent Office, which states: "biological activity may include immunogenic activity of the full length protein." (See Response paper dated 05 August 1998, filed by patent applicant.)". Associate Professor Rogers then states:

> "Immunogenicity is not generally considered to be a "biological activity" of a protein, because the term "biological activity" is generally used to describe the functions of a protein in native host cells or organisms where the protein does not normally cause an antibody response."

4.46 This is a specious statement. I would include immunological activity within the context of what is understood by the phrase biological function or activity. The ability of a protein to be able to bind an antibody regardless of its origin is, in my opinion, clearly a biological interaction and an activity that is dictated by the specific primary construction of the protein. Such activity is in my opinion a *bona fide* activity of a protein, since the antibodies may be used for clinical purposes, such as to inhibit a VEGF-2 mediated ailment or in a diagnostic manner such as to identify VEGF-2 activity. Thus immunological activity may be a biological function or activity that is dictated by the amino acid sequence of the identified protein.

4.47 Further, in paragraph 2.7.4 Associate Professor Rogers states:

> "...short peptide sequences of 5, 6, 7, or more residues could be considered biologically active fragments of VEGF-2, because fragments of this size are generally considered large enough to

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elicit an immune response... Both VEGF-2 and the prior art human VEGF polypeptide contain an identical 7-mer sequence RCGGCCN... Thus, polynucleotides that encode VEGF satisfy the "encodes VEGF-2 or a biologically active fragment of VEGF-2" limitation of claims 13-15..."

4.48 At the conclusion of this paragraph Associate Professor Rogers identifies Documents D6 (p. 523 Fig. 1), D7 (p. 16319 Fig. 1), D12, D18 (p. 1307 Fig. 1A and 1B), and D34 to D36 as publications that anticipate claims 13 to 15. None of these documents establish that the amino acid sequence RCGGCCN preferentially binds a VEGF-2 antibody. Likewise document D29 (cited in footnote 8) which describes Balbiani Ring 3 Protein does not identify an antibody that preferentially binds VEGF-2 antibody. I refer to and repeat my comments in paragraphs 4.31 to 4.34, above.

Paragraph 2.7.5

4.49 In paragraph 2.7.5 Associate Professor Rogers states:

"Claims 16-18 are directed to an isolated polynucleotide which hybridizes to a VEGF-2 polynucleotide and which encodes a polypeptide which binds an antibody capable of binding to VEGF-2. As explained above in paragraphs 2.4-2.5, polynucleotides which encode VEGF satisfy the hybridizing limitation and encode a polypeptide (VEGF) which satisfies the antibody binding limitation."

4.50 I disagree. I refer to and repeat my comments in paragraphs 4.28 to 4.30, above, which address these matters in more detail.

Paragraph 2.7.12

4.51 In paragraph 2.7.12 Associate Professor Rogers states:

"As explained above in paragraphs 2.4-2.4.3, prior art polynucleotides that encode prior art VEGF polypeptides (or BR3P polypeptides) will hybridize to the VEGF-2 polynucleotides or fragments thereof recited in the claims. As explained in paragraphs 2.5 and 2.7.4, VEGF (and probably BR3P) polypeptides described in the prior art bind antibodies which bind to VEGF-2. Thus, VEGF polypeptides (and BR3P polypeptides) satisfy all of the limitations of claims 40-45, so these claims are anticipated by prior art documents that disclose VEGF (and BR3P) polypeptides."

4.52 In my opinion the conclusions reached by Associate Professor Rogers in this paragraph are not accurate. When I read the word "hybridize" in those claims I understand it to mean that the hybridization reaction

should be conducted under suitable conditions such as those described in Example 1 and stated on page 6. lines 2-5 of the specification. In Example 1, the patent specification teaches hybridization conditions that would not allow for cross hybridization of unrelated sequences, particularly VEGF or BR3P. These conditions are at either 65 degree C at 0.2xSSC or 60 degree C with 0.5XSSC and 0.1% SDS. Thus, the claims do not, in my opinion, encompass VEGF or BR3P polynucleotide sequences.

Paragraph 2.7.18

4.53 In paragraph 2.7.18 Associate Professor Rogers states:

> "...the claims directed to antagonists of VEGF-2 are not novel over prior art disclosures of forms of the receptors to which VEGF-2 could bind, but could not signal. See **Document D27** (disclosing a dominant negative Flk-1 protein)."

4.54 I can find no evidence in document D27 which establishes that the dominant negative Flk-1 protein described could bind VEGF-2. I observe that the dominant negative Flk-1 receptor described in D27 is created by deleting a significant portion of the intracellular kinase binding domain from part of one of the Flk-1 proteins that forms the receptor. Such a deletion may well inhibit binding of VEGF-2. In my opinion, no conclusions can be drawn from D27 about whether a dominant negative Flk-1 protein might serve as a VEGF-2 antagonist.

4.55 Separately claim 50 is directed to VEGF-2 antagonists. I note that that claim reads as follows:

> "An antagonist specific for the polypeptide according to any one of claims 28 to 48"

4.56 I would understand the words "specific for" as used in this claim to mean that the antagonist must bind specifically to a VEGF-2 polypeptide and not one of the then known (i.e. in 1994) prior art polypeptides. Thus to the extent that Associate Professor Rogers refers to the Flk-1 receptor as a possible molecule that binds VEGF-2 I would note that it preferentially binds to VEGF and thus is not specific for VEGF-2. Hence I would not understand the Flk-1 receptor to fall within the scope of claim 50.

Paragraph 2.7.19

4.57 In paragraph 2.7.19 Associate Professor Rogers states:

> "...patients in need of VEGF-2 (or in need of inhibiting VEGF-2) would be treatable with VEGF (or with VEGF antagonists). Consequently, claims 51 and 52 embrace any prior art method of treatment of patients with VEGF polypeptides that are encompassed by claim 28 (or prior art method of treatment with VEGF antagonists)."

4.58 When I read the claims in the patent specification I note that they relate to a method of treating a patient in need of VEGF-2 using a VEGF-2 protein. I would not understand claims 51 to 52 to embrace a method of treating patients with VEGF, because the claims appear to me to be limited to the use of one or more VEGF-2 molecules.

Paragraph 2.7.21

4.59 In paragraph 2.7.21 Associate Professor Rogers states:

> "Since the Figures also disclose VEGF and PDGF's (See, *e.g.* Figures 2A-2B), I find that this reference to the Figures does not serve to exclude the prior art, and claims 57-61 are also anticipated."

4.60 I do not agree with Associate Professor Rogers' comments. I note that each of claims 57 to 61 are limited to earlier claims which specifically define VEGF-2 subject matter. In my opinion none of the identified claims encompass VEGF or PDGF. Hence I do not believe that the prior art molecules are covered by these claims.

Paragraph 3.4.2

4.61 In paragraph 3.4.2 Associate Professor Rogers states:

> "The specification also fails to provide any teaching or exemplification of active fragments, analogues, and/or derivatives of VEGF-2."

4.62 Separately, in paragraph 2.7.20 Associate Professor Rogers stated:

> "...the opposed application fails to identify with particularity any fragment, analogue, or derivative of the Figure 1 polypeptide which has an inhibitory activity."

4.63 I do not agree that the specification fails to describe fragments of VEGF-2. I refer to Example 2 in the patent specification where HGS describe a VEGF-2 fragment (see page 29 lines 17 to 24 of the patent specification).

Further, at page 5 (lines 31 to 35) the patent specification describes two portions of VEGF-2, namely, the boxed area in Figure 2 which corresponds to amino acids 61 to 74 and the region bound by the cysteine residues which corresponds to amino acids 38 to 118.

4.64 Producing fragments, analogues or derivatives of a known sequence in 1994 was a routine task that I would expect any researcher of ordinary skill in the field of molecular biology would have been able to accomplish by employing standard and routine trial and experimentation. In this respect I refer to paragraph 3.2.5 of Associate Professor Rogers' Statutory Declaration where he makes the following comments:

> "The common general knowledge at the time of the filing of the priority application included knowledge of the following:
>
> .....
>
> materials and methods for generating short peptides of any desired amino acid sequence synthetically;
>
> materials and methods for selectively altering one or more codons of a gene and using the altered gene to produce proteins with one or more amino acids selectively changed or deleted or added;..."

4.65 Further, at paragraph 3.5 Associate Professor Rogers states:

> "Before the 1994 priority date, it was routine for the skilled addressee to introduce 3-4 amino acid (or codon) changes into a polypeptide (or polynucleotide) sequence, *e.g.* using procedures such as site-directed mutagenesis. [See, *e.g.* documents cited in paragraph 3.2.2, above.]."

4.66 These statements by Associate Professor Rogers are consistent with my comments about the routine nature of preparing VEGF-2 fragments, analogues or derivatives using the information in the patent specification.

4.67 Once an analogue fragment or derivative of VEGF-2 had been prepared it would then have been a matter of testing that molecule for the biological function or activity. Such activity could have been tested using any one or more of the *in vivo*, *in vitro* or immunological systems described in the patent specification. I refer to paragraphs 4.18 to 4.24 above which discuss the availability of such assays.

4.68 I believe the patent specification provides all of the information that I would have required to identify a fragment, analogue, or derivative of the Figure 1 polypeptide which had an inhibitory activity. Likewise, the

patent specification contains all of the information that I would have required to produce active fragments, analogues, and/or derivatives of VEGF-2. I would not have needed (in 1994) examples in the patent specification before I could have produced or identified them and or tested them in a biological assay.

Paragraph 3.7.1

4.69 In paragraph 3.7.1 Associate Professor Rogers states:

> "I find no description of any instances where VEGF-2 gene therapy was successfully performed, or even attempted. Also, I find no teachings or guidance relating to performing gene therapy treatment that were not within the common general knowledge in Australia at the time of filing the opposed application."

4.70 The description of gene therapy provided in the patent specification is in my opinion suitably descriptive of the process involved. The success of such an approach would depend on the use of external technologies such as those used to deliver the VEGF-2 gene.

4.71 Had I wanted to identify publications describing gene therapy approaches as at March 1994 I would have conducted a Medline search. Some publications providing examples where gene therapy had been successfully used prior to March 1994 are illustrated below.

4.71.1 Stewart C, Taylor NA, Docherty K, Bailey CJ (1993) "Insulin delivery by somatic cell gene therapy." *J. Mol. Endocrinol.* 11:335-341.

4.71.2 Walsh CE, Liu JM, Miller JL, Nienhuis AW, Samulski RJ (1993) "Gene therapy for human hemoglobinopathies." *Proc. Soc. Exp. Biol. Med.* 204:289-300.

4.71.3 Kay MA, et al. (1993) "In vivo gene therapy of hemophilia B: sustained partial correction in factor IX-deficient dogs.". *Science* 262:117-119.

4.71.4 Kolodka TM, Finegold M, Kay MA, Woo SL (1993) "Hepatic gene therapy: efficient retroviral-mediated gene transfer into rat hepatocytes in vivo." . *Somat. Cell Mol. Genet.* 19:491-497.

4.71.5 Hyde SC, et al. (1993) "Correction of the ion transport defect in cystic fibrosis transgenic mice by gene therapy." *Nature* 362:250-255.

4.71.6 Goldspiel BR, Green L, Calis KA (1993) "Human gene therapy." *Clin. Pharm.* 12:488-505.

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4.72 Had I wanted to carryout VEGF-2 gene therapy I would have consulted both the description of gene therapy provided in the patent specification and one or more gene therapy publications, such as those list above.

4.73 Given the information in the patent specification and the state of the literature so far as it pertained to gene therapy by 1994 I believe that the description provided in the patent application is sufficient for me to perform experiments leading to gene therapy treatment.

Paragraph 4.6.4

4.74 In paragraph 4.6.4 Associate Professor Rogers states:

> "...the applicant and inventors failed to predict what appears to be one of the most important activities of VEGF2, that of a growth factor for the lymphatics system."

4.75 The patent specification states that VEGF-2 can be used in the promotion of endothelialization (see for example page 4 lines 11 and 12, page 17 and 18 of the patent specification). By 1994, the lymphatic system was known to be composed of endothelial cells. Moreover the intrinsic growth characteristics of lymphatic endothelium and vascular endothelium were reported to share similarities. Some publications reporting this knowledge are illustrated below:

4.75.1 Yong LC, Jones BE (1991) "A comparative study of cultured vascular and lymphatic endothelium" *Exp. Pathol.* 42:11-25; and

4.75.2 Witte MH, Witte CL (1987) "Lymphatics and blood vessels, lymphangiogenesis and hemangiogenesis: From cell biology to clinical medicine" *Lymphology* 20: 257-266.

Paragraph 4.7

4.76 In paragraph 4.7 Associate Professor Rogers states:

> "A molecular biologist would not have expected a partial eukaryotic polynucleotide sequence to be properly expressed in eukaryotic cells if the sequence were missing the first 69 codons."

4.77 Had I been presented with the patent specification at 1994 I would have attempted to produce the protein as described in the patent specification. In the process of attempting to do so, one of the options that I would have employed would have been to express the protein using a heterologous secretion signal sequence as described in the patent specification. If I

had thought that it was possible the sequence presented in the patent specification was in fact a truncated form of a larger molecule, I would have also attempted to clone more of the 5' end of the gene. Hence I would have followed the teachings in the patent specification and I would have looked further upstream for additional coding sequence for the full-length molecule.

4.78 In view of the above I do not agree with the comments of Associate Professor Rogers. I believe that using routine trial and experimentation a scientist of ordinary skill would have been able to produce the biologically active protein identified as VEGF-2 in the patent specification in eukaryotic cells even if the sequence were missing the first 69 codons.

Paragraph 4.8

4.79 In paragraph 4.8 Associate Professor Rogers states:

> "The failure to demonstrate a VEGF-2 activity and the failure to provide a VEGF-2 activity assay and the suggestion that VEGF-2 may have several activities (uses) place an undue burden on the part of the skilled addressee to practice the invention."

4.80 I disagree with this statement. In my opinion the patent specification provides sufficient information to permit a person of ordinary skill to practice the invention. I refer to and repeat paragraphs 4.18 to 4.24 above, which highlight some of the information provided in the patent specification.

Paragraph 4.10

4.81 In paragraph 4.10 Associate Professor Rogers refers to claims 16-27, 40-50 and 57-61 and suggests that the subject matter claimed in those claims was not intended by HGS to be part of the invention. I do not agree with that suggestion nor on my reading does the patent specification (see pages 4 and 22 to 24 where HGS provide a variety of suggested uses for which the defined fragments may be used).

4.82 Further, Associate Professor Rogers indicates there is no suggestion by HGS that antibodies had been made at the time the patent specification was filed. Regardless of whether HGS had actually produced any antibodies at the time of filing the patent specification, on page 23 there is a disclosure identifying how to produce such antibodies, which in my opinion any person of ordinary skill could have followed to generate

VEGF-2 antibodies. It was not necessary that the patent specification establish that HGS had made antibodies, before I could have taken the disclosed amino acid sequence and used it to generate such antibodies.

4.83 Associate Professor Rogers also suggests that HGS has not identified all of the antigenic sites on the VEGF-2 molecule. Although computer programs where readily available in 1994 to generate this information, I would not have needed such information. To determine whether a protein sequence would bind a VEGF-2 antibody is simply a matter of testing it. Additionally, as described in the specification, one routine way to generate antibodies is to inject isolated protein into a rabbit, which causes the rabbit to naturally raise antibodies against VEGF-2. In this method, it is not necessary to know the antigenic sites in order to generate the antibodies. Nothing further is required and any researcher in this field could have identified such binding.

Paragraph 4.13.1

4.84 In paragraph 4.13.1 Associate Professor Rogers states:

> "The opposed application reports that a message of 1.6 kD was observed in Northern hybridization studies. Initially, I note that scientists do not normally report the size of mRNA in kiloDaltons (kD, a measurement of molecular weight), but rather, report such sizes in length, *e.g.* kilobases (kb)."

4.85 From reading the patent specification it is clear to me that the reference to kD in Example 1 is a typographical error and should in fact be read as kb. No other possible explanation makes sense. This reasoning is also consistent with the information presented in Figure 5.

4.86 Further, in paragraph 4.13.1 Associate Professor Rogers states:

> "I observe that the description of the experimental results in the text of the specification does not correspond with the results depicted in the Figure. There is no significance whatsoever ascribed to the results allegedly depicted in Figure 5. Figure 5 appears to depict hybridization of a VEGF-2 probe to an mRNA species identified as 1.3 kb in size, which is inconsistent with results reported in Example 1 (Figure 4 and text) and inconsistent with other studies."

4.87 Figure 5 illustrates that VEGF-2 may be isolated from a range of different tissues.

4.88 Associate Professor Rogers comments on the band of mRNA species at 1.3kb. I would regard the VEGF-2 sequence identified in the patent specification that has a 1.6kb molecular weight to be within the range of error for the 1.3 kb band identified in Figure 5. Experiments of the nature presented in Example 1 and Figure 5 are not highly accurate. Further, strict alignment is not observed between the molecular weight markers and the molecular weight identification on the other side of the gel. Thus I do not believe that this result necessarily reflects that VEGF-2 was not isolated.

Paragraph 4.13.3

4.89 In paragraph 4.13.3 Associate Professor Rogers comments on Example 2. Before turning to Associate Professor Roger's comments I would like to make some observations about Example 2.

4.90 When I read Example 2 I noted that there are a number of errors in the Example that appear to me to be typographical in nature. First, Example 2 fails to identify the sequence of VEGF primer F5. I will discuss this issue in more detail below.

4.91 Second, Example 2 on page 29, line 21 refers to lanes 1 and 3, however this reference is inconsistent with the results presented in Figure 6. I believe the reference to lanes 1 and 3 should read lanes 3 and 5, which is consistent with the Figure 6 data. As reported in the legend associated with figure 6, lane 1 represents molecular weight markers. Thus lane 1 can not possibly represent a PCR product of full length VEGF-2 cDNA. Further the reference to lane 2 at line 24 should read lane 4. This would have been obvious to me and I believe a person of ordinary skill in my field, when regard is had to Figure 6.

4.92 Third, I note that the F4 primer is located about 119 bp away from the 3'end of the stop codon and about 242 bp before the last nucleotide of the cDNA. Further reference to the F4 primer should I believe be a reference to the F5 primer since it binds the VEGF sequence outside the VEGF-2 ORF. Thus the patent specification fails to provide the F4 primer. The specification states however that the F4 primer binds the nucleotide sequence approximately 36 amino acids before the COOH end of the VEGF-2 ORF. Since the specification provides the nucleotide sequence in this locality I and I believe any other researcher of ordinary skill in the field could have used Figure 1 to generate a suitable primer

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that would lead to the production of a VEGF-2 sequence truncated by 36 amino acids at its COOH terminus.

Paragraph 5.6

4.93 In paragraph 5.6 Associate Professor Rogers states:

> "Many of the claims (*e.g.*, at least claims 1-4, 11, 13-20, 28, 32, 34-45, and certain claims that depend therefrom) seek protection well beyond any consideration provided in the application to the extent that they can be interpreted to read on non-human forms of the VEGF-2 polynucleotide or polypeptide or uses thereof."

4.94 The patent specification teaches to me the existence of VEGF-2 generally and the information provided in the patent specification is sufficient, in my opinion, to identify and isolate VEGF-2 from other mammalian and animal species using standard and routine probing or PCR procedures that were commonly available in 1994. Hence I do not regard the patent specification to be limited to human VEGF-2. Any person of ordinary skill in the field could have used the information in the patent specification to produce probes and / or primers, which could have been used to identify VEGF-2 from another mammalian and animal species.

Paragraph 5.8

4.95 In paragraph 5.8 Associate Professor Rogers refers to a number of the claims in the patent specification that specifically define individual fragments of VEGF-2. I am aware that there is an error in the numbering of the amino acids in SEQ ID NO: 2. The amino acid sequence designated one (1) to five (5) (inclusive) actually constitutes amino acids one (1) through six (6) (inclusive). When this error is taken into account, all of the amino acid numbering appears to me to be correct.

Paragraph 5.8.1

4.96 In paragraph 5.8.1 Associate Professor Rogers comments on claims 11 and 32 in the patent specification. In that paragraph he states:

> "...it remains my opinion that the specification evinces no intent *to claim* a genus of polypeptides using this sequence as the critical, defining limitation. The context of the quoted statement indicates that the statement is merely one of comparison of the structural-relatedness of the VEGF-2 sequence to prior art sequences. There is no mention of the exact sequence *per se*, but rather, merely an observation that VEGF-2 includes a segment that

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matches a motif (PXCVXXXRCXGCCN, where X is any amino acid)."

4.97 I disagree with Professor Rogers where he suggests that there is no mention of the exact sequence *per se*. Immediately after the identification of the signature motif on page 5 of the patent specification there is a reference to Figure 2. In Figure 2 the signature motif is defined as a boxed region between amino acids 61 to 74, wherein the exact sequence of the signature motif is defined. Thus, in my view, the patent specification does define the signature motif.

4.98 I note that Associate Professor Rogers makes a comment that in his opinion there is no intention in the patent specification to claim polypeptide sequences with the signature motif as the limiting feature. The patent specification by way of the disclosure on page 5 and Figure 2 clearly identifies the signature motif. Further, the patent specification on page 9 makes clear to me that it includes fragments of VEGF-2. Given that the identified signature motif is conserved between VEGF family members I would have understood that the identified signature motif is one of the fragments that is talked about on page 9 of the specification. Thus the patent specification does support fragments bounded by amino acid residues 61 to 74.

Paragraphs 5.8.2 & 5.8.3

4.99 In paragraph 5.8.2 and 5.8.3 Associate Professor Rogers discusses claims 34 and 40. For similar reasons I believe the patent specification provides support for claims 11 and 32 I also believe that it provides support for claim 34 and 40. I refer to and repeat my comments in paragraphs 4.94 to 4.96, which address this matter.

Paragraph 5.8.4

4.100 In paragraph 5.8.4 Associate Professor Rogers refers to claims 12 and 33 and states:

> "There is only mention of the cysteines, and no mention of the intervening 70+ amino acids that are dispersed between the cysteines and that comprise most of the sequence between 37 and 117."

4.101 I do not agree with this statement. The patent specification on page five identifies the eight cysteine residues as being of importance in the overall structure of VEGF-2. Further SEQ ID No: 2 and Figure 1 identify each of

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the subject cysteine residues, including all of the intervening residues. I believe that all of the necessary information to produce this sequence as a fragment of VEGF-2 is provided in the patent specification. Moreover page 5 of the patent specification makes clear to me that HGS identified the sequence bound by amino acids 37 to 117 as being a significant portion of the disclosed VEGF-2 sequence.

Paragraphs 5.8.5 & 5.8.6

4.102 In paragraph 5.8.5 and 5.8.6 Associate Professor Rogers discusses claims 35 and 41. To the same extent that I consider the patent specification provides support for claims 12 and 33, it also provides support for claim 35 and 41. I refer to and repeat my comments in paragraphs 4.98 to 4.99, which address this matter.

Paragraph 5.8.10

4.103 In paragraph 5.8.10 Associate Professor Rogers refers to claim 39 and states:

> "As explained above in detail, Example 2, which provides the only arguable description of VEGF-2 mRNA, apparently was performed incorrectly, and VEGF-2 mRNA was misidentified .....Thus, I find no support in the application for this term, either."

4.104 I disagree. I refer to page 4 of the patent specification, which discloses RNA polynucleotides. Additional disclosures of such subject matter may be found on page 6 of the specification and in Example 1, where I note the applicant actually identified a number of VEGF-2 mRNA species. The probe that was used in the hybridization reaction described in Example 1 was a VEGF-2 sequence as disclosed in the patent specification. Having regard to the hybridization conditions used in Example 1 I believe that the results presented in Figure 5 support the identification of VEGF-2 mRNA, which therefore means that such mRNA can be isolated using routine and conventional PCR and cDNA techniques.

Paragraph 5.9

4.105 In paragraph 5.9 Associate Professor Rogers states:

> "When one considers what is now known about VEGF-2, it becomes apparent that the consideration provided in the specification is little more than a partial sequence with no demonstration of activity."

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4.106 I disagree that the patent specification provides little more than a partial sequence with no demonstration of activity. In my opinion the patent specification provides all of the essential information required to identify and produce a biologically active VEGF-2 as well as guidance as to the uses of that protein. I refer to paragraphs 4.3 to 4.24 above which highlight the information contained in the patent specification.

Paragraph 6.6

4.107 In paragraph 6.6 Associate Professor Rogers states:

> "Because the definition of "activity" is unclear, the definitions of "antagonist" and "inhibitory activity" cannot be ascertained."

4.108 When I read the patent specification I note that it describes, for example, at least an activity of VEGF-2 as being an ability to proliferate endothelial cells. Other activities such as wound healing or as inhibitors of tumours, etc. are also discussed. Thus, I would understand an antagonist of VEGF-2 to be something that interferes with one or more of the biological activities of VEGF-2 as illustrated in the patent specification. Such terms were well known and well understood by researchers of ordinary skill in the field by 1994.

Paragraph 6.8.2

4.109 In paragraph 6.8.2 Associate Professor Rogers refers to claims 51 and 54 and states:

> "Claims 51 and 54 (and claims dependent therefrom) are ambiguous in that each apparently intends that a therapeutic amount of a polypeptide can be administered without administering any polypeptide whatsoever. In particular, claim 54 specifies that the therapeutically effective amount of polypeptide is administered by providing DNA to a patient. DNA is not a polypeptide, but a polynucleotide. Thus, even though claims 51 and 54 on their face recite administration of a polypeptide therapeutic, the applicant apparently intends for these claims to encompass gene therapy that does not involve administering *any* of the indicated polypeptide. Thus, the meaning of "therapeutically effective amount of the polypeptide" is unclear, and apparently includes an amount equal to zero."

4.110 When I read claims 51 and 54 I understand them to be referring to a method of treating a patient by administering to that patient a therapeutically effective amount of a VEGF-2 polypeptide. Two means are defined for administering the amino acid sequence. In the first

method (claim 51) the polypeptide is administered directly to the patient. In the second method (claim 54) the polypeptide is delivered via a process of DNA expression *in vivo*. In each instance the amount of protein that can be delivered to a patient can be controlled to give a specific therapeutic effect. The delivery of therapeutically effective amounts of a polypeptide can be empirically determined applying routine trial and experimentation.

4.111 When Associate Professor Rogers states: "...claim 54 specifies that the therapeutically effective amount of polypeptide is administered by providing DNA to a patient. DNA is not a polypeptide, but a polynucleotide...", I do not know what he means. As I read the claims I would not understand them to be suggesting that DNA is a polypeptide. In my opinion the claims require the administration of a DNA sequence in a manner permitting expression of a VEGF-2 polypeptide and nothing more.

4.112 In my opinion this subject matter is clearly supported by pages 18 and 19 in the patent specification. Moreover using the general information in the patent specification and nothing more than routine trial and experimentation a person of reasonable skill in the field could have practiced the method defined by claim 54.

AND I make this solemn declaration by virtue of the Statutory Declarations Act, 1959 and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DATED this 12th day of December 2000.

DECLARED at: Brisbane, Queensland)

BEFORE me:.....)

.....

JOHN STANLEY MATTICK

K Brodrick (J.P.Qual)

~~Commissioner of Declarations/Patent Attorney~~/Justice of the Peace/~~Solicitor~~

Kellie Ann Brodrick
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BARDON QLD 4065

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of:
Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

Annexure JSM-1

This is **Annexure JSM-1** referred to in my Statutory Declaration made this 12th day of DECEMBER 2000.

John Stanley Mattick

WITNESS: K. Brodrick (J.P. Qual) 7707

~~Commissioner for Declarations/Solicitor~~
~~Patent Attorney~~/Justice of the Peace

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JOHN STANLEY MATTICK

Professor of Molecular Biology
Director, Australian Genome Research Facility
Director, Special Research Centre for Functional and Applied Genomics
Co-Director, Institute for Molecular Bioscience
The University of Queensland, Brisbane, Australia 4072

BIOGRAPHICAL SUMMARY

John Mattick was born in Sydney in 1950 and was educated at St Patrick's College, Strathfield, where he was Dux. He received his B.Sc with First Class Honours in Biochemistry from the University of Sydney in 1972, followed by a PhD in Biochemistry in 1977 from Monash University, on the topic of mitochondrial DNA replication and mutation. He then undertook postdoctoral work on the molecular biology of the fatty acid synthetase complex at the Baylor College of Medicine in Houston Texas from 1977 to 1981. His work on the architecture and function of this complex is now standard in biochemistry textbooks. He returned to Australia in early 1981 to work as a research scientist at the (then) CSIRO Division of Molecular Biology in Sydney, where he was the leader of a team responsible for the development of one of the world's first genetically engineered vaccines (against ovine footrot), for which he was awarded the 1989 Pharmacia-LKB Biotechnology Medal from the Australian Society for Biochemistry and Molecular Biology.

In 1988 he was appointed Professor of Molecular Biology and Foundation Director of the Centre for Molecular Biology and Biotechnology at the University of Queensland. The Centre was subsequently made a Special Research Centre of the Australian Research Council, and in 1994 was renamed the Centre for Molecular and Cellular Biology. The Centre has grown rapidly and has a strong national and international reputation for its research on the molecular biology of mammals and their diseases, including gene mapping, gene regulation, and developmental and cell biology. The Centre has over 140 staff and research students, and in 2000 was integrated into a new Institute for Molecular Bioscience (IMB), which is currently being constructed as part of a $110m research complex in conjunction with CSIRO. Professor Mattick is Co-Director of the IMB, and Director of a new ARC Special Research Centre for Functional and Applied Genomics, which was also established in 2000. Professor Mattick is also the Director of the Australian Genome Research Facility which was established under the Major National Research Facilities Program in 1987 with a $10m grant from the Australian Government, and which has its headquarters and DNA sequencing division at the University of Queensland, and its genotyping and mutation detection division at the Walter and Eliza Hall Institute of Medical Research in Melbourne.

Professor Mattick is an advocate of research into the information content of genes, including the Human Genome Project. He has worked in Sydney, Melbourne, Houston, Brisbane, Cambridge and Oxford, on viruses, bacteria and mammals, including protein chemistry, cell culture, and gene cloning and expression. His current research interests include the molecular genetics of host infection by type 4 fimbriate bacterial pathogens such as *Pseudomonas aeruginosa*, the analysis of the *P. aeruginosa* genome, the molecular biology of mammalian embryogenesis, and the role of introns and RNA processing in the evolution and development of multicellular organisms. He has advanced

the radical notion that introns and other so-called "junk" RNA molecules produced by the higher organisms represent a parallel processing system for gene control, the evolution of which was the critical step in the emergence 500 million years ago of sophisticated multicellular organisms from the unicellular life forms that had occupied the Earth's biosphere for the preceding 3 billion years. He has published over 100 papers in molecular biology, and was awarded the Eppendorf Achievement Award at the 2000 Lorne Genome Conference for his services to Australian Molecular Biology.

Professor Mattick has been a member of the Board of the Australian National Genome Information Service (ANGIS) since its inception in 1991. He is a member of the Human Genome Organisation (HUGO) and was the Chairman of the Organising Committee of the 1999 Human Genome Meeting (HGM'99) held in Brisbane, and a member of the Scientific Committees of the 1998 Human Genome Meeting (Turin) and the 2000 Human Genome Meeting (Vancouver). Since 1997 he has been a member of the Australian Health Ethics Committee and the Research Committee of the National Health and Medical Research Council, and over the past 12 years has served on numerous NHMRC Regional Grants Interviewing Committees and *ad hoc* Committees of Review for Program and Institutional Block Grants, as well as on the advisory boards of several organisations, including the Wellcome Trust Australian Medical Research Fellowships, the John Curtin School of Medical Research, the Mater Medical Research Institute and the Australian Proteome Analysis Facility. He is currently a member of the Executive of BIOCOG (Biotechnology Consultative Group) which advises the Federal Minister for Industry, Science and Resources, and is a member of the Queensland Biotechnology Advisory Council.

CURRICULUM VITAE

JOHN STANLEY MATTICK

PERSONAL

Date of Birth: 26th April, 1950
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Family: Married to Louise Ellen O'Gorman
Three sons (John, born 1988; James, born 1997; Angus, born 2000)

DEGREES AND AWARDS

Undergraduate: B.Sc. (First Class Honours in Biochemistry) 1968-1971
The University of Sydney

Postgraduate: Ph.D. 1972-1977
"The replication and maintenance of mitochondrial DNA in *Saccharomyces cerevisiae*"
Department of Biochemistry, Monash University

Postdoctoral Pharmacia-LKB Biotechnology Medal,
Australian Biochemical Society 1989

Butland Visiting Professorship, The University of Auckland 1997

Eppendorf Achievement Award, Lorne Genome Conference 2000

PROFESSIONAL HISTORY

1977-1981	Department of Biochemistry, Baylor College of Medicine Houston, Texas, U.S.A.
1977-1979	*Research Associate*
1979-1981	*Member of Faculty (Instructor)*

1982-1988	CSIRO Division of Molecular Biology, Sydney, NSW
1982-1984	*Research Scientist*
1984-1987	*Senior Research Scientist*
1987-1988	*Principal Research Scientist*

1988-present	The University of Queensland, Brisbane, QLD
1988-present	*Professor of Molecular Biology, Department of Biochemistry*
1988-1990	*Director, Centre for Molecular Biology and Biotechnology*
1991-1993	*Director, ARC Special Research Centre for Molecular Biology and Biotechnology*
1994-1999	*Director, ARC Special Research Centre for Molecular and Cellular Biology*
1996-present	*Director, Australian Genome Research Facility*
2000-present	*Director, ARC Special Research Centre for Functional and Applied Genomics*
2000-present	*Co-Director, Institute for Molecular Bioscience*

Sep 1993 - Mar 1994	Visiting Scientist, Department of Genetics, The University of Cambridge, Cambridge UK
Mar 2000 - Sep 2000	Visiting Senior Research Fellow, St. John's College, and Department of Anatomy and Human Genetics The University of Oxford, Oxford UK

CURRENT PROFESSIONAL ACTIVITIES – partial list

- Member, Research Committee of the National Health and Medical Research Council
- Member, Australian Health Ethics Committee of the National Health and Medical Research Council
- Member, BIOCOG (Biotechnology Consultative Group, reporting to the Federal Minister for Industry, Science and Resources)
- Member, Board of the Australian National Genome Information Service
- Member, Board of the Australian Proteome Analysis Facility
- Member, Board of the Mater Medical Research Institute
- Member, Research Advisory Board of the John Curtin School of Medical Research

- Member, Board of Genset Pacific Pty. Ltd. and Australian Genome Diagnostics Pty. Ltd.
- Member, International Molecular Biology Network (IMBN) Asia-Pacific
- Member, Queensland Biotechnology Advisory Council

CURRENT RESEARCH INTERESTS

- The molecular genetics of host colonisation by type 4 fimbriate bacterial pathogens
- Analysis of the *Pseudomonas aeruginosa* genome
- RNA-binding proteins and gene expression during mammalian development
- RNA-based gene regulation and the role of introns in multicellular development
- Genome sequencing and genome evolution as an information system

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PATENTS

1. ***Antigenic Preparation***
   (Australian Patent No. 34979/84)
   Cloning and expression of *Bacteroides nodosus* fimbrial subunit genes in heterologous hosts as a basis for production of new vaccines against footrot.
   Inventors: **J.S. Mattick** and B.J. Anderson
   Filed: Australia and New Zealand 2-11-84.

2. ***Improved Antigenic Preparation***

(Australian Patent No. 50154/85; International PCT No. W086/02557)
Morphogenetic expression of fimbrial subunit genes in *Pseudomonas aeruginosa*, or other compatible hosts, as a basis for the production of fimbrial antigens for vaccines against footrot and other type 4 fimbriate pathogens.
Inventors: **J.S. Mattick**, B.J. Anderson and T.C. Elleman
Filed: Australia, U.S.A., Europe, South Africa and New Zealand 29-10-85.

3. ***Peptide Production by Protein Engineering***

(Australian Patent No. 17049/88; International PCT No. W088/08430)
Use of type 4 fimbrial subunits as a vehicle for the expression and export in recombinant bacterial cells of peptide sequences, such as the FMDV VP1 epitope 144-159, introduced by oligonucleotide-directed gene mutagenesis.
Inventors: **J.S. Mattick** and P.A. Jennings
Filed: Australia, U.S.A., Europe 27-4-87.

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46.* **Mattick, J.S.** (1995) A-Z is not enough: the molecular genetics of type 4 fimbriae in *Pseudomonas aeruginosa* and related pathogens. The 3rd Australian Conference on Molecular Analysis of Bacterial Pathogens, Marysville, Victoria.

47.* **Mattick, J.S.** (1995) Intron-exon structure and the evolution of parallel processing. Michael J. D. White Memorial Lecture, Genetics Society of Australia, Canberra, ACT.

48.* Alm, R.A. and **Mattick, J.S.** (1995) Genes involved in the biogenesis and function of type 4 fimbriae in *Pseudomonas aeruginosa*. Workshop on Type 4 pili - biogenesis, adhesins, protein export, and DNA import, Schloß Ringberg, Germany.

49.* **Mattick, J.S.** (1996) Genome research - the information superhighway of biotechnology. 10th International Biotechnology Symposium, Sydney, NSW. Abstract S6-5, p. 42.

50. Wood, S.A. and **Mattick, J.S.** (1996) Fam is a novel ubiquitin-specific protease expressed during postimplantation mouse development. Australian and New Zealand Society for Cell Biology Meeting, Brisbane.

51.* **Mattick, J.S.** (1996) Genome research and drug discovery. 2nd Australia-Japan Symposium on Drug Design and Development, Cairns, Queensland.

52.* **Mattick, J.S.** (1997) The Australian Genome Research Facility. 2nd International Strategy Meeting on Human Genome Sequencing, Hamilton, Bermuda.

53. Whitchurch, C.B., Darzins, A., Alm, R.A., Hobbs, M., Martin, P.R., Nourse, C.R., Watson, A. and **Mattick, J.S.** (1997) *Pseudomonas aeruginosa* genes *pilL*, *chpA* and *chpB* are necessary for type 4 fimbrial biosynthesis and twitching motility. 97th General Meeting of the American Society of Microbiology, Miami USA. Abstract D-41, p. 214.

54.* **Mattick, J.S.**, Whitchurch, C.B., Martin, P.R., Alm, R.A. and Hobbs, M. (1997) Regulatory pathways affecting the biogenesis and function of type 4 fimbriae in *Pseudomonas aeruginosa*. VI International Meeting on *Pseudomonas*: Molecular Biology and Biotechnology, Madrid, Spain.

55.* **Mattick, J.S.**, Alm, R., Whitchurch, C.B., Martin, R. and Hobbs, M. (1998) The molecular genetics of type 4 fimbriae in *Pseudomonas aeruginosa* and related bacterial pathogens. 9th National BiotechnologySeminar, Penang, Malaysia.

56. Comolli, J., Waite, L., Hauser, A., Whitchurch, C., **Mattick, J.** and Engel, J. (1998) Pilin function is necessary for *Pseudomonas aeruginosa* cytotoxicity and invasion of epithelial cells. 98th General Meeting of the American Society of Microbiology, Abstract B-81, p. 69.

57.* **Mattick, J.S.** (1998) Genomics in Australia. Conference on Biological Informatics, Australian Academy of Science, Canberra.

58.* **Mattick, J.S.** (1998) The implications of the human genome project for medicine and healthcare in the 21st century. Australian Pharmaceutical Manufacturers Association Conference, Canberra.

59.* **Mattick, J.S.** (1998) Genomics and genetics in drug discovery and drug delivery. Third Australia / Japan Symposium on Drug Design and Development, Tokushima, Japan.

60.* **Mattick, J.S.** (1998) Intron function and eukaryote evolution. Queenstown 1998 Molecular Biology Meeting, Queenstown, New Zealand.

61.* **Mattick, J.S.** (1998) Genomics and genetics - from bacteria to humans. Joint Meeting of the New Zealand Microbiology Society and The New Zealand Society for Biochemistry and Molecular Biology, Masterton, New Zealand.

62.* **Mattick, J.S.** (1998) Host colonisation by *Pseudomonas aeruginosa*. Joint Meeting of the New Zealand Microbiology Society and The New Zealand Society for Biochemistry and Molecular Biology, Masterton, New Zealand.

63.* Beatson, S.A., Whitchurch, C.B. and **Mattick, J.S.** (1998) Whole genome analysis of *Pseudomonas aeruginosa*: identification of novel genes and pathways. Joint Meeting of the New Zealand Microbiology Society and The New Zealand Society for Biochemistry and Molecular Biology, Masterton, New Zealand.

64. L. Croft, B. Huang, R. Blakeley, S. Beatson, C.B. Whitchurch and J.S. Mattick (1999) Annotation of the *Pseudomonas aeruginosa* PA01 genome. Human Genome Meeting 1999 (HGM'99), Brisbane.

65.* Croft, L., Whitchurch, C.B., Beatson, S., Blakeley, R., Huang, B. and **Mattick, J.S.** (1999) Exploring the *Pseudomonas aeruginosa* genome. Abstract S31. *Pseudomonas'99*: biotechnology and pathogenesis. American Society for Microbiology, Maui, Hawaii.

66.* Whitchurch, C.B., Young, M., Leech, A., Semmler, A. and **Mattick, J.S.** (1999) The molecular genetics of type 4 fimbriae and twitching motility in *Pseudomonas aeruginosa*. Abstract S41. *Pseudomonas'99*: biotechnology and pathogenesis. American Society for Microbiology, Maui, Hawaii.

67. Clark, F., Croft, L., Schandorff, S., Burrage, K. and Mattick, J.S. (1999) ISIS – the intron database: a glimpse of intron function. Abstract S-18-03. Combio '99 (43rd Annual Meeting of the Australian Society for Biochemistry and Molecular Biology, 18th Annual Meeting of the Australian and New Zealand Society for Plant and Developmental Biology, and the 39th annual Meeting of the Australian Society of Plant Physiologists), Gold Coast.

68. Croft, L., Beatson, S.A., Whitchurch, C.B., Blakeley, R., Huang, B. and **Mattick, J.S.** (1999) One approach to developing a bacterial genome database. Abstract S-25-02. Combio '99 (43rd Annual Meeting of the Australian Society for Biochemistry and Molecular Biology, 18th Annual Meeting of the Australian and New Zealand Society for Plant and Developmental Biology, and the 39th annual Meeting of the Australian Society of Plant Physiologists), Gold Coast.

69. Kennedy, H.D., French, J., Verhagen A. and **Mattick, J.S.** (1999) The *ras*-GTPase-activating protein SH3-domain-binding family of proteins (G3BPs): implications in *ras*-GAP^{120} signalling to RNA stability and cancer progression. Abstract S-33-03. Combio '99 (43rd Annual Meeting of the Australian Society for Biochemistry and Molecular Biology, 18th Annual Meeting of the Australian and New Zealand Society for Plant and Developmental Biology, and the 39th annual Meeting of the Australian Society of Plant Physiologists), Gold Coast.

70. Utama, B., Kennedy, H.D., Ru, K. and **Mattick, J.S.** (1999) Isolation and preliminary characterization of a novel nucleolar protein. Abstract P-W-51. Combio '99 (43rd Annual Meeting of the Australian Society for Biochemistry and Molecular Biology, 18th Annual Meeting of the Australian and New Zealand Society for Plant and Developmental Biology, and the 39th annual Meeting of the Australian Society of Plant Physiologists), Gold Coast.

71.* Croft, L., Whitchurch, C.B., Beatson, S., Blakeley, R., Huang, B. and **Mattick, J.S.** (1999) Exploring the *Pseudomonas aeruginosa* genome. Abstract OP-A28, p. 433. 11th National Biotechnology Seminar, Melaka, Malaysia.

72. Croft, L., Schandorff, S., Clark, F., Burrage, K., Arctander, P. and **Mattick, J.S.** (2000) ISIS, an intron information system, and the prevalence of alternative splicing in the human genome. Human Genome Meeting 2000 (HGM'2000), Vancouver, Canada.

73. **Mattick, J.S.** (2000) After the human genome project: implications for medicine, healthcare and humanity in the 21st century. The 11th International Congress of Endocrinology, Sydney.

RECENT LECTURES / RESEARCH SEMINARS

1. *The new genetics and the story of life.* The Queensland Museum, 1991.
2. *The scientific and commercial implications of genome projects and genome analysis.* The 10th Australian Biotechnology Conference, Melbourne, February 1992.
3. *The significance of genome research.* International Genome Science Meeting, Adelaide, February 1992.
4. *Microbial genomes.* International Genome Science Meeting, Adelaide, February 1992.
5. *The new genetics and zoological conservation.* The Second Joint ARAZPA/ASZK Annual Zoological Conference, Currumbin Sanctuary, April 1992.
6. *Genome analysis - a paradigm shift in biological research.* The Campus Genetics Society, University of New South Wales, April 1992.
7. *The new genetics and the story of life.* The 1992 Butler Memorial Lecture, University of Queensland, September 1992.
8. *A genome approach to exploring biological systems.* Fairfield Hospital, Melbourne, July 1993.
9. *The molecular genetics of type 4 fimbriae in bacterial pathogens.* Department of Genetics, Cambridge University, Cambridge, September 1993.
10. *The Gene Library.* Department of Biochemistry and Molecular Biology, UMIST, Manchester, October 1993.
11. *Does one gene equal one protein?* Department of Genetics, Cambridge University, Cambridge, March 1994.
12. *Does one gene equal one protein: the significance of introns and RNA-based gene regulation in eukaryotic evolution.* Adelaide Children's Hospital, Adelaide, June 1994
13. *Does one gene equal one protein: the significance of introns and RNA-based gene regulation in eukaryotic evolution.* Childrens Medical Research Institute, Sydney, June 1994.
14. *Does one gene equal one protein: the significance of introns and RNA-based gene regulation in eukaryotic evolution.* Department of Biochemistry, University of Queensland, Brisbane, August 1994.
15. *Does one gene equal one protein: the significance of introns and RNA-based gene regulation in eukaryotic evolution.* Queensland Institute for Medical Research, Brisbane, August 1994.
16. *The new genetics?* Genetic Futures: the scientific, ethical, social, religious and environmental implications of genetic technology. Conference held by The Australian

Institute of Ethics and the Professions at St Johns College, the University of Queensland. September 1994.

17. *Biogenesis of type 4 fimbriae in Pseudomonas aeruginosa and related pathogens.* Department of Microbiology and Immunology, The University of Adelaide, Adelaide, October 1994

18. *Graduation Address* The University of New South Wales Faculties of Science and Medicine, Sydney, October 1994.

19. *Does one gene equal one protein: the role of introns in eukaryotic development.* The John Curtin School of Medical Research, Australian National University, Canberra, October 1994.

20. *The 1995 Colliver Lecture: The new genetics - implications for our future.* The Queensland Museum, Brisbane, June 1995.

21. *The biogenesis, function and regulation of type 4 fimbriae, and their relationship to other virulence factors in Pseudomonas aeruginosa.* Institute of Molecular Medicine, University of Oxford, Oxford, November 1995.

22. *The importance of genome research to medicine.* Australian Medical Students Association 1996 Meeting, Brisbane, June 1996.

23. *RNA signalling and processing in mammalian development.* The Institute for Reproduction and Development, Monash Medical Centre, July 1996.

24. *The impact of molecular genetics on the future of chemistry.* 14th International Conference on Chemical Education, Brisbane, July 1996.

25. *RNA signalling and processing in mammalian development.* Johnson and Johnson Research Centre, Sydney, August 1996.

26. *Genomes and development.* Faculty of Science, Queensland University of Technology, Brisbane, September 1996.

27. *The Australian Genome Research Facility.* Peter MacCallum Institute for Cancer Research, Melbourne, September 1996.

28. *The Australian Genome Research Facility.* Genetics, Cancer and Cardiovascular Disease Conference, Lorne, September 1996.

29. *The human genome project.* Royal Society of Queensland Special Symposium *on Exploring our genes and genetic heritage*, Brisbane, October 1996

30. *Understanding the human genome.* XIIIth Annual Royal North Shore Hospital/ University of Technology Sydney Scientific Research Meeting, Sydney, November 1996.

31. *The impact of genome projects on medical research and the future of medicine.* AWT Edwards Oration at the 35th National Scientific Conference of the Australian Society for Medical Research, Gold Coast, November 1996.

32. *The information age in biotechnology.* The Licensed Executives Society (Australia and New Zealand), Brisbane, March 1997.

33. *Butland Visiting Professor Oration: The human genome project and the future of medicine.* The University of Auckland, Auckland, August 1997.

34. *RNA-mediated gene regulation in mammalian development.* Faculty of Health Sciences, University of Auckland, Auckland, August 1997.

35. *Type 4 fimbriae.* Faculty of Health Sciences, University of Auckland, Auckland, August 1997.

36. *The human genome project and the changing face of biology.* CONSTAQ '97 (1997 Conference of the Science Teachers Association of Queensland), Brisbane, August 1997.

37. *The human genome project and the future of biology.* The Leo Howard Vacation School, University of Queensland, Brisbane, January 1998.

38. *The impact of genomics on the future of biology and medicine. Human Genome Research - Science and Society.* The Garvan Institute of Medical Research, Sydney, April 1998.

39. *Genomics and biotechnology.* NZ Medical Research Council Foresight Meeting, Auckland, June 1998.

40. *Genomics in Australia.* Bioinformatics Conference, Australian Academy of Science, Canberra, July 1998.

41. *The Institute of Molecular bioscience.* University of Queensland Customs House, Brisbane, November 1998.

42. *Genomics.* 9th Wheat Breeding Assembly, Toowoomba, September 1999.

43. *The impact of genomics on the future of medicine.* Australian College of Legal Medicine, Canberra, October 1999.

44. *Novel therapies for the new millenium.* Leukaemia Foundation Annual Conference, QIMR, Brisbane, October, 1999.

45. *Genomics.* Biofutures Conference, The Brisbane Institute, Brisbane, October 1999.

46. *Host colonisation by Pseudomonas aeruginosa – complex signal transduction pathways which integrate multiple virulence factors and colonial behaviour.* Children's Medical Research Institute, Westmead, Sydney, October 1999.

47. *The end of reductionist biology.* The Queensland Protein Group, Brisbane, November 1999.

48. *Twitching motility in bacteria.* Department of Plant Sciences, The University of Oxford UK, May 2000.

49. *The molecular genetics of type IV pili and host colonisation in Pseudomonas aeruginosa.* Department of Cell and Molecular Biology, Umeå University, Umeå Sweden, June 2000.

50. *The role of introns and RNA-based gene regulation in eukaryotic evolution and development.* Department of Human Anatomy and Genetics, The University of Oxford UK, June 2000.

51. *The development of the Institute for Molecular Biosciences and the future of biology as an information science.* School of Biomedical Sciences, University of Ulster, Coleraine UK, June 2000.

52. *The molecular genetics of type IV fimbriae and host colonisation in Pseudomonas aeruginosa and construction of an interactive web-based genome database.* Department of Microbiology and Immunobiology, Queens University, Belfast UK, June 2000.

53. *The implications of the human genome project for medicine, healthcare and humanity in the 21st century.* The Kuringai District Medical Association and South African Medical Association, Sun City, South Africa, July 2000.

54. *Striking the balance in Australia: "A Code of Ethical Practice for Biotechnology".* Australian Biotechnology Event, World Expo 2000, Hannover, Germany, July 2000.

55. *Biomedical, Biotechnology and Pharmaceutical Innovation: Australia's Opportunities.* Australian Biotechnology Event, World Expo 2000, Hannover, Germany, July 2000.

56. *The role of introns and RNA-based gene regulation in eukaryotic evolution and development.* EMBL (European Molecular Biology Laboratory), Heidelberg, Germany, July 2000.

57. *The molecular genetics of type IV fimbriae and host colonisation in Pseudomonas aeruginosa and construction of an interactive web-based genome database.* Department of Medical Microbiology, St Bartholomew's and the Royal London School of Medicine and Dentistry, London, UK, July 2000.

58. *The molecular genetics of type IV fimbriae and host colonisation in Pseudomonas aeruginosa and construction of an interactive web-based genome database.* Lehrstuhl Biologie der Mikroorganismen, Ruhr-Universitaet Bochum, Bochum, Germany, September 2000.

59. *The molecular genetics of type IV fimbriae and host colonisation in Pseudomonas aeruginosa and construction of an interactive web-based genome database.* Institute for Molecular Medicine, Oxford, UK, September 2000.

60. *The evolution of controlled multitasked networks: a role for introns and other noncoding RNAs.* CNRS Marseille, France, September 2000.

61. *The molecular genetics and genomics of Pseudomonas aeruginosa host colonisation and pathogenesis.* Austin Research Institute, Melbourne, November 2000.

62. *The modern RNA world: tips of an iceberg.* Ludwig Institute for Cancer Research, Melbourne, November 2000.

SCIENTIFIC AND ACADEMIC SERVICES

Scientific Journals:

- Infection and Immunity - Reviewer
- Gene - Reviewer
- Molecular Microbiology – Reviewer
- Microbiology
- Journal of Bacteriology - Reviewer
- FEMS Microbiology Reviews – Reviewer
- Australian Medical Journal - Reviewer
- Asia-Pacific Journal of Molecular Biology and Biotechnology - Editorial Board

The University of Queensland

- Member, Academic Board 1989-present
- Member, Faculty of Science 1989-1996
- Member of the Executive, Faculty of Chemical and Biological Sciences 1997-present
- Member, Faculty of Medicine / Health Sciences 1990-1996

Other Institutions

- Member, NHMRC Regional Grants Interviewing Committee 1988-92, 1994,1998
- Chairman, NHMRC Regional Grants Interviewing Committee 1992, 1994, 1998
- Member, NHMRC Assigners Panel 1990-95
- Member, NHMRC C.J.Martin Fellowships Referee Panel 1990
- Member, Australian Wool Corporation Genetic Projects Review Group 1990
- Member, Selection Committee for the Australian National Genome Information Service 1990
- Member, NHMRC Fogarty Fellowships Committee 1991-92
- Member, Executive and Board of the Australian Genome Information Centre 1991-present
- Member, Biomedicine Advisory Board of the Australian Nuclear Science and Technology Organisation 1991-1994
- Member of Organizing Committee, the Xth Australian Biotechnology Conference, Gold Coast, Queensland, 1991
- Organizer/Convener, the 14th Annual Conference on the Organization and Expression of the Genome, Lorne, Victoria, 1992
- Foundation Director, Lorne Genome Conference Inc. 1992-1997
- Member, Review Committee of the Molecular Parasitology Program, CSIRO Division of Tropical Animal Production 1992
- Member, Quinquennial Review Committee of the Garvan Institute for Medical Research 1992
- Member, Hamilton NHMRC Program Grant Review Committee 1992
- Member, NHMRC Genome Working Party 1991-93
- Chair, NHMRC Genome Working Party 1994-96
- Member, Martin NHMRC Program Grant Review Committee, 1995
- Member, Joint Review of the John Curtin School of Medical Research, The Australian National University, 1995
- Member of Organizing Committee, the 8th International Conference on Isozymes - Gene Families: Structure, Function, Genetics and Evolution. Brisbane, Queensland, 1995
- Member of International Advisory Committee, the 10th International Biotechnology Symposium. Sydney, New South Wales, 1996
- Member of Council, Royal Society of Queensland, 1996-1997
- Member, Scientific Advisory Committee, Cooperative Research Centre for Vaccine Technology, 1996-1997
- Organizer/Convener, the 19th Annual Conference on the Organization and Expression of the Genome, Lorne, Victoria, 1997
- Member, Review Committee of Research at the Royal Adelaide Hospital, IMVS and the Hanson Centre, 1997
- Member, Board of Pacific Oligos Pty. Ltd., 1997-1999

- Member, Advisory Board of the John Curtin School for Medical Research, The Australian National University, 1997-2000
- Member, Board of the Cooperative Research Centre for The Discovery of Genes for Common Human Diseases. 1997-1999
- Member, Research Committee of the National Health and Medical Research Council, 1997-2003
- Member, NHMRC Australian Health Ethics Committee, 1997-2003
- Member, Research Review Committee of The Prince Charles Hospital, Brisbane, 1998
- Foundation Member. Asia-Pacific International Molecular Biology Network (IMBN), 1998 - present
- Member, Organising Committee of HGM'98 (Human Genome Meeting), Turin 1998
- Chair, Organising Committee of HGM'99 (Human Genome Meeting), Brisbane 1999
- Chair, Initial Quinquennial Review of the Victor Chang Heart Research Institute, 1999
- Member, Organising Committee, IXth International Congress of Bacteriology and Applied Microbiology, Sydney, 1999
- Member, Board of the Mater Medical Research Institute, 1999 - present
- Member, Program Committee ASM Pseudomonas '99 Conference, Maui, Hawaii, 1999
- Member, Board of the Australian Proteome Analysis Facility, 1999 – present
- Member, Advisory Panel. Universiti Kebangsaan Malaysia – Malaysia Technology Development Corporation (UKM-MTDC) Biotechnology Academy, 1999 – present
- Co-Editor (with Prof. Paul Davies), Frontiers of Science series (Allen & Unwin), 1999 - present
- Member, Scientific Advisory Board, Medica Holdings Pty. Ltd., 1999 – present.
- Member, Executive of Biotechnology Consultative Group (BIOCOG), 1999 – present
- Member, Organising Committee of HGM'2000 (Human Genome Meeting) Vancouver, 2000
- Member, Board of Pacific Oligos Pty. Ltd., 2000 – present
- Member, Board of Australian Genome Diagnostics Pty. Ltd., 2000 – present
- Member, Scientific Advisory Committee of the Clive and Vera Ramaciotti Centre for Gene Function Analysis, University of New South Wales, 2000 – present
- Member, Queensland Biotechnology Advisory Council, 2000 - present

MEMBERSHIP OF PROFESSIONAL SOCIETIES

- The Australian Society for Biochemistry and Molecular Biology
- The Australian Society for Microbiology
- The Genetics Society of Australia
- The Lorne Genome Conference

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of:
Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

Annexure JSM-2

This is **Annexure JSM-2** referred to in my Statutory Declaration made this 12th day of DECEMBER 2000.

John Stanley Mattick

WITNESS: K. Broderick (J.P. Qual) 7727

~~Commissioner for Declarations/Solicitor~~
~~Patent Attorney~~/Justice of the Peace

Kellie Ann Broderick
143 Forrester Tce
BARTON QLD 4065

Summary Research History

John Stanley MATTICK

1.1 My training in molecular biology and biochemistry began in 1968 when I undertook an honours degree in science at the University of Sydney, Australia. My honours thesis investigated the control of cyclopropane fatty acid synthesis in *Pseudomonas denitrificans*.

1.2 After receiving a first class honours degree in 1972, I moved to the Department of Biochemistry at Monash University in Melbourne, Australia to undertake Ph.D. studies on the replication and maintenance of mitochondrial DNA in *Saccharomyces cerevisiae* (baker's or brewer's yeast, which is widely studied as a genetic model for cellular function). Between 1972 and 1977, I investigated replicative DNA synthesis in *Saccharomyces cerivisiae* mitochondria (see publications 2 and 3) and the effects of inhibitors and mutagens, such as ethidium bromide, acridines and their derivatives, on the replication and maintenance of the mitochondrial genome (see publications 1, 4, 5 and 6). In 1977, I received a Ph.D. for my research from Monash University.

1.3 In 1977, I moved to the United States of America to work as a Post-Doctoral Fellow in the Department of Biochemistry at Baylor College of Medicine in Houston, Texas. In the 1970s and early 1980s, Baylor College of Medicine was one of the leading centres for molecular biology in the world, and remains so today. At Baylor my research interests took on a new focus: an examination of the fatty acid synthetase complex, which is the enzyme system used to synthesise fats for membranes and for energy storage. Using molecular biology techniques, my colleagues and I isolated and cloned, for the first time, the fatty acid synthetase gene complex from yeast (see publication 11). I also purified messenger RNA (mRNA) encoding the corresponding mammalian and avian enzyme complexes (see publications 7 and 8) and developed a new system for the *in vitro* translation of mRNA into protein (see publication 10).

1.4 I also made monoclonal antibodies against the fatty acid synthetase complex and used these and other reagents, in conjunction with controlled proteolysis, to dissect its structure and architecture and to map the location of its active sites. This research was published in four consecutive papers in the Journal of Biological Chemistry (see publications 12, 13, 14 and 15) and provided a clear insight into the

mechanism by which the fatty acid synthetase protein complex works in animals, which is now the standard treatment in major biochemistry textbooks. We showed that fatty acid synthetase is a multi-enzyme complex that consists of a head-to-tail dimer of two identical polypeptide chains, which are encoded by a single gene. Each polypeptide chain is multi-functional in activity and contains all seven enzymatic functions and the acyl carrier required to synthesise lipids from 2-carbon compounds. This research provided me with experience in many of the techniques associated with gene cloning and expression including RNA isolation, mRNA purification, *in vitro* translation, cDNA synthesis, recombinant library construction and methods for screening recombinant libraries.

1.5 In 1981, I returned to Australia to take up a position as a Research Scientist with the (then) Commonwealth Scientific and Industrial Research Organisation ("CSIRO") Division of Molecular Biology in Sydney. CSIRO was interested in using my expertise to develop new recombinant DNA-based vaccines against important pathogens. At their request, I began by investigating the prospects for developing a vaccine against the blood-borne protozoan parasite *Babesia bovis* (which is related to malaria) (see publication 16), but the immunological basis for such a vaccine was unclear and this research was discontinued. During this time, however, I was also involved in the successful cloning, sequencing and identification by translation of hybrid-selected mRNAs of the cDNA sequences encoding the type-specific surface glycoprotein of rotavirus (which causes diarrhoeal disease) (see publications 17 and 18).

1.6 In 1983, I started working on the development of a recombinant DNA-derived vaccine against the bacterium *Bacteroides (*now *Dichelobacter) nodosus*, which causes footrot in ruminants, and for which the important protective antigens (called type 4 fimbriae) were known. I achieved this objective by isolating and cloning the gene sequences encoding these antigens from *Bacteroides nodosus*, expressing the corresponding proteins from those sequences and finally testing the proteins for their efficacy as a vaccine. The initial gene cloning component of my research was completed by the end of 1984, and the selection of a suitable bacterial host cell (*Pseudomonas aeruginosa*) to obtain correct folding and secretion of the protein, selection of a suitable expression vector and testing of a prototype vaccine, was completed by the end of 1985 (see publications 19, 20, 22, 30 and 33, and patents 1 and 2). This

represented the first successful genetically-engineered vaccine in Australia and one of the first in the world. For this work the Australian Biochemical Society subsequently awarded me the 1989 Pharmacia-LKB Biotechnology Medal.

1.7 During this time also, my (then) wife was concurrently employed as a Research Scientist at Biotech Australia Pty. Ltd. and was the leader of the successful project to clone and express human plasminogen activator inhibitor type 2, by constructing cDNA libraries and screening with oligonucleotide probes derived from partial amino acid sequence information. I closely followed and was familiar with her work on this project.

1.8 By about the end of 1985, I supervised a team of about 10 researchers, including a Post-Doctoral Fellow, Ph.D. students, CSIRO research assistants, and seconded employees of collaborating vaccine and biotechnology companies.

1.9 Between 1985 and 1988, my colleagues and I cloned the genes encoding related antigens from other *Bacteroides nodosus* serotypes to expand the range of coverage of the vaccine, and to improve the host-vector expression system to enable stable high-level production of these proteins for vaccine manufacture. This required optimisation of the promoter system to drive high level expression of the recombinant protein, selection of suitable vector systems that were stable in the host cell during large-scale fermentation, suitable splice sites for insertion of the cloned gene into the expression vector, and appropriate procedures for purification of the protein from recombinant culture (see publications 23, 24, 25, 26, 27, 29, 31, 32, 37, 42, 45, 46 and 56).

1.10 My team also examined other antigenic proteins and showed that the recombinant fimbrial expression system could be successfully used to manufacture vaccines against other bacterial pathogens, such as *Moraxella bovis* (which causes severe conjunctivitis and eye damage in cattle), and to produce antigenic epitopes from other organisms such as foot-and-mouth disease virus by site directed mutagenesis and protein engineering (see publications 36 and 41).

1.11 During this period I also worked as a consultant for the Mauri Foods Pty Ltd yeast genetic engineering program. I was also involved, then and

later, in the characterisation of important genes and proteins from bovine herpes viruses and enteroviruses (see publications 34, 43, 44, 63, 66 and 91).

1.12 In 1988, I was appointed the Foundation Professor of Molecular Biology and the Foundation Director of the Centre for Molecular Biology and Biotechnology ("the Centre") at the University of Queensland, positions that I continue to hold. I established and built the Centre and in 1990 it was designated a Special Research Centre of the Australian Research Council (formerly known as Commonwealth Centres of Excellence), one of only about twenty in all areas of science and one of only four in biology. The Centre was re-named the ARC Special Research Centre for Molecular and Cellular Biology in 1994. It specialises in the molecular genetics, developmental biology and cellular biology of mammals and their pathogens, and currently has over 130 staff and research students with an annual budget of around $7 million.

1.13 The laboratories in the Centre, including my own, routinely use recombinant expression systems in bacteria, yeast, insect cells (baculovirus) and animal cells to produce recombinant proteins for purposes such as x-ray crystallography and other forms of structural analysis, to generate antibody probes for various experimental purposes, and to study the biochemistry and interactions of such proteins with other proteins, RNA and DNA.

1.14 Since 1988, my research has focussed primarily on the molecular genetics of the biogenesis and function of type 4 fimbriae in a number of pathogenic bacteria, but particularly in *Pseudomonas aeruginosa* (which causes opportunistic infections in immuno-compromised individuals such as those suffering from cystic fibrosis, AIDS, burns and cancer chemotherapy) (see publications 28, 31, 35, 40, 46, 47, 51, 61, 62, 64, 65, 68, 69, 71, 72, 74, 75, 76, 77, 79, 81, 82, 83, 84, 85, 86, 87, 94, 95, 98, and 100).

1.15 I have also developed a program to identify genes involved in mammalian development and the role of RNA in this process (see publications 73, 78, 88, 89, 90, 92, 97, 99, 101, 102), as well as a number of new techniques in molecular biology (see publications 49, 60 and 67), and collaborative projects in mammalian gene cloning and mapping (see publications 48, 54, and 57). This involved, among other things, gene identification,

bioinformatic analysis and gene expression in heterologous expression systems to obtain antibodies to analyse functional roles of the encoded proteins *in vivo*. My colleagues and I have also been involved in various collaborative projects in gene expression and protein engineering. This has involved cloning of bacterial and mammalian genes, site-directed mutagenesis to alter the coding sequence and consequently the protein product, expression of the product and functional assays (see publications 52, 53, 58, 59, 88, and 101). My laboratory has also developed new vector systems for gene cloning and recombinant expression in bacterial cells (see publication 79). I have also been an active proponent of genome research in Australia (see publications 38, 39, 55, 70, 80 and 93).

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of:
Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

Annexure JSM-3

This is **Annexure JSM-3** referred to in my Statutory Declaration made this 12th day of DECEMBER 2000.

J. J. Mattick

John Stanley Mattick

WITNESS: K. Broderick (J.P. Qual.) 77707

~~Commissioner for Declarations/Solicitor~~
~~Patent Attorney/~~Justice of the Peace

Kellie Ann Broderick
1/43 Forrester Tce
BARTON QLD 4065

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

DOCUMENT LIST

Documents provided to me by the Patent Attorneys representing HGS in the subject proceedings are as follows:

1. Passaniti A, et al. (1992) *Lab. Invest.* 67:519-528.
2. Splawinski J, et al (1988) *Methods Find Exp. Clin. Pharmacol.* 10:221-226.
3. Andersson et al. (1992) *J. Biol. Chem.* 267:11260-11266.
4. Betsholtz et al. (1986) *Nature* 320:695-699.
5. Breier et al. (1992) *Development* 114:521-532.
6. Claffey et al. (1992) *J. Biol. Chem.* 267:16317-16322.
7. Ferrara et al. (1992) *Endocrine Rev.* 13:18-32.
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27. US patent 6,130,071

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of:
Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

Annexure JSM-4

This is **Annexure JSM-4** referred to in my Statutory Declaration made this 12th day of DECEMBER 2000.

John Stanley Mattick

WITNESS: K. Broderick / J.P. Grant 7727

~~Commissioner for Declarations/Solicitor~~
~~Patent Attorney/~~Justice of the Peace
Kellie Ann Broderick
1/43 Forrester Tce
BARDON QLD 4065

Table 1

Australian Patent Application 696764 (73941/94)

In the name of: Human Genome Sciences Inc.

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, **Nicholas Kim Hayward** of The Human Genetics Laboratory, Queensland Institute of Medical Research, Herston, QLD 4028, Australia, a research scientist, declare as follows:

1. Professional History.

1.1. I am currently Head of the Human Genetics Laboratory, Queensland Institute of Medical Research (QIMR) I have held this position since 1987. I am also currently an NH&MRC Senior Research Fellow, a Senior Research Fellow at the Queensland Centre for Schizophrenia Research, Wolston Park Hospital, Chairman of the Joint Experimental Oncology Programme of the QIMR, the University of Queensland and the Queensland Cancer Fund, and a conjoint lecturer at the Department of Pathology, University of Queensland.

1.2. Now produced and shown to me marked "**NKH-1**" is a copy of my curriculum vitae, which itemises the publications and presentations of which I have been an author or co-author.

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1.3. I received a Ph.D. in biochemistry from The University of Queensland in 1983. From 1983 to 1990, I was an NH&MRC Research Officer/Senior Research Officer at QIMR, during which time I mapped and identified human genes involved in cancers, such as melanoma, using linkage analysis and positional cloning techniques.

1.4. In 1991, I was awarded an NH&MRC R. D. Wright Fellowship for three years. Following a lecture at QIMR given by Dr J. Shepherd from The Department of Surgery, University of Tasmania, Hobart in about 1989, I became involved in trying to find the gene responsible for multiple endocrine neoplasia type 1 (MEN 1), again using linkage studies and positional cloning techniques. This involved searching the region of human chromosome 11, to which the MEN 1 locus was known to map, for putative genes, identified as open reading frames (ORFs) in DNA clones that we obtained and sequenced. Each candidate ORF that we identified was studied, to determine the possible function of the protein encoded by the ORF. Assigning putative function to each ORF then allowed us to consider whether the ORF was likely to be involved in a suitable physiological pathway that could lead to MEN 1.

1.5. One cDNA clone that we identified early by 1994, although not involved in MEN 1, was determined by us to be highly related to a growth factor known as vascular endothelial growth factor (VEGF). Analysis of the corresponding genomic DNA indicated that the gene encoded two splice variants, one of 167 amino acids and one of 186 amino acids. Careful analysis of these variants revealed that they belonged to the PDGF/VEGF gene family. As of 1994, I was aware of the PDGF/VEGF family of proteins although, until this finding, I was not working with this family.

1.6. After further analysis, I concluded that the gene that I had newly identified was a member of the PDGF/VEGF family of growth factors and was most closely related to VEGF. Consequently I termed the polypeptide encoded by the gene, VEGF-related factor (VRF). This was subsequently changed to VEGF-B in accordance with international nomenclature.

1.7. The results of the cloning work and subsequent sequence analysis that my colleagues and I conducted were published in Grimmond *et al.*, 1996, Genome Research 6: 124-131.

1.8. After isolating human VEGF-B, I went on to clone the corresponding murine orthologue of VEGF-B, including the genomic sequence as well as the cDNA sequence. This work was published in Townson *et al.*, 1996, Biochemical and Biophysical Research Communications 220: 922-928. I also conducted further work to identify and analyse the promoter region of human VEGF-B, which was published in Silins *et al.*, 1997, Biochemical and Biophysical Research Communications 230: 413-418.

1.9. My involvement with VEGF-B is ongoing: I am currently collaborating with Drs Kay and Mould to study the biological role of VEGF-B in mouse development, pathology and neoplasia using, for example, gene knock-out mice defective in VEGF-B. Some of this work has been reported in Bellomo *et al.*, 2000, Circulation Research 86: e29-e35, where it is shown that VEGF-B expression is temporally and spatially regulated, suggesting a role for VEGF-B in ventricular growth.

1.10. In addition to my interest in VEGF-B, during the remainder of the 1990s I continued to work in the field of human cancer genetics, publishing a further 50 scientific papers in this area between 1994 and 1999.

1.11. Thus, I am familiar with the background knowledge, experience and technical abilities of researchers in my field, especially in Australia over the last fifteen years through my research, writing, supervisory responsibilities and referee duties for various international journals. I have been required to develop and maintain a good knowledge of Australian and international scientific literature for a diverse range of scientific fields including molecular biology, biochemistry and cell biology etc. I am very familiar with the field of human genetics and the cloning and analysis of human genes over the last fifteen years. I have published about 100 scientific papers, almost all of which have been concerned with molecular biology and human cancer genetics. In particular, I have direct experience in cloning a member of the PDGF/VEGF family.

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1.12. In the following sections I refer to various scientific publications and patent specifications. Unless otherwise identified, I have not enclosed copies of these documents with this statutory declaration since the patent attorneys representing Human Genome Sciences Inc ("HGS") have informed me that copies of these documents will be filed by separate means.

2. My Instructions

2.1 I am informed by the Patent Attorneys representing HGS that these proceedings concern an opposition by Ludwig Institute for Cancer Research to Australian Patent specification AU-B-696764 (73941/94) by HGS, entitled "Vascular Endothelial Growth Factor 2" ("the patent specification") which has an earliest date of filing of 8 March 1994 ("March 1994"). I have been asked to provide my comments and opinions on the patent specification for use in these proceedings. I have also been asked to provide my comments and opinions as to what the patent specification would provide to one of ordinary skill in the field of molecular biology as of March 1994. My opinions concerning the content (information) in the patent specification are contained in this statutory declaration.

2.2 When I first met the Patent Attorneys for HGS I was provided with a copy of a document entitled "*Guidelines for Expert Witnesses in Proceedings in the Federal Court of Australia*."

2.3 The Patent Attorneys for HGS have provided me with copies of numerous documents. Now produced and shown to me marked "**NKH-2**" is a list of those documents. I have been asked to review these documents and to provide my comments thereon. I do not propose to address each and every paragraph in the statutory declarations that I have reviewed. This decision of mine should not be taken as an admission on my part of acceptance of any text that I do not comment on.

3. Specific comments concerning the patent specification

3.1 In the following paragraphs I comment on the information contained in the patent specification so far as it is relevant to the issues that have been broadly addressed by one or more of Ludwig Institute for

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Cancer Research's expert witnesses. In the next section of this statutory declaration I will provide specific responses to some of the salient paragraphs from Associate Professor Rogers' and Dr Alitalo's statutory declarations.

The meaning of the term "VEGF-2"

3.2 The patent specification provides the nucleotide sequence and amino acid sequence of a 350 amino acid polypeptide, the sequences being shown in Figure 1. This polypeptide is designated VEGF-2. In addition, the cDNA clone that was sequenced to obtain the nucleotide and amino acid sequences shown in Figure 1 has been deposited and in my opinion could readily be sequenced by a skilled person to obtain VEGF-2 sequence information. To be concise, I will confine my comments essentially to polypeptides since the polynucleotide claims are generally limited to sequences that encode VEGF-2 polypeptides and so essentially the same comments apply.

3.3 In biological terms, a protein is governed by its primary amino acid sequence, which determines the structure and function of the protein. However, a protein may exist in a variety of forms that do not have exactly the same primary amino acid sequence but are nonetheless recognisable from the primary amino acid sequence as being the same protein. For example, there may be allelic variants that differ between individuals by a few amino acids. In addition, there may be equivalent proteins in other organisms that have a substantial number of amino acid differences but are nonetheless recognisable as being essentially the same protein (known as orthologues) and not a different protein.

3.4 Consequently, the term VEGF-2 conveys to me the idea of not just the sequences presented in the patent specification, but a family of related sequences. Once a protein has been identified any given sequence can be tested to determine whether any amino acid changes affect the structural and or functional characteristics of that protein.

3.5 Some examples of fragments described in the patent specification include the mature protein lacking the proprotein portion (page 6 third paragraph) and the protein exemplified in Example 2, which is

missing 36 amino acids from its carboxy end. Other fragments could have been produced by 1994 with routine ease using the information in the patent specification.

3.6 The patent specification states that VEGF-2 is involved in angiogenesis (pages 16-17), to promote endothelialisation (page 4 third paragraph and page 17 fourth full paragraph) or tumour angiogenesis and/or tumour neovascularization (page 17 last full paragraph to page 18), as a wound healing agent (page 16 last paragraph that extends to page 17), to treat heart attacks (myocardial infarctions) and cell death due to the loss of blood (ischaemia) (page 17 third full paragraph), and for *in vitro* culturing of vascular endothelial cells (page 18 first paragraph). I therefore consider active fragments to include fragments of VEGF-2 that might for example have one or more of these activities. Alternatively they may have other angiogenic activities that could have been tested using any of the then routinely available assays. Suitable assays were well known by 1994 for determining angiogenic activities including the above activities. For example, *in vitro* three dimensional gel assays could have been used to measure the angiogenic properties of VEGF-2, as reviewed and described in Cockerill, *et al.*, 1994, In: International Reviews of Cytology. A Survey of Cell Biology 159: 113-160 and cell proliferation assays could have been used to measure the mitogenic properties of VEGF-2, as described in Maglione *et al.*, 1991, Proceedings of the National Academy of Sciences (USA) 88: 9267-9271.

3.7 In summary, I consider the phrase "a VEGF-2 polypeptide having the deduced amino acid sequence of Figure 1 or an active fragment, analogue or derivative of said polypeptide" to mean not only the actual human sequence given in Figure 1 but also similar sequences. These sequences may be cloned from other organisms and variants that occur naturally, in the case of allelic variants, or have been produced by mutagenesis procedures (non-naturally occurring variants) as well as chemically modified derivatives and fragments of the above of a suitable size such that they have VEGF 2 biological function or activity, such as *in vivo*, *in vitro* or immunological activity.

NA

3.8 However, I do not believe, as is asserted by Associate Professor Rogers, that the phrase "fragments, analogues or derivatives" encompasses PDGF, VEGF and PIGF polypeptides or polynucleotides. Specifically, I do not consider PDGF, VEGF or PIGF to be analogues or derivatives of VEGF-2, as each of these proteins are distinct, with each having their own unique amino acid sequence as shown in Figure 2. Further, I do not believe that this view would be shared by a skilled person. The patent specification specifically distinguishes PDGF, VEGF and PIGF, providing a sequence comparison between these three prior art proteins and VEGF-2 and giving their percentage homology to one another (see page 5 last full paragraph and Figure 2 of the specification). In particular I note that there is not sufficient sequence identity between VEGF-2 and PDGF, VEGF and PIGF (as is clearly demonstrated in Figure 2) for these molecules to be considered derivatives of VEGF-2. This provides me with further confirmation that the claims should not be taken to include these proteins.

Polynucleotides - hybridisation

3.9 A number of the claims of the HGS application refer to nucleotide sequences that hybridise to the nucleotide sequence shown in the sequence listing (SEQ ID No. 1) or the cDNA deposited in a specific ATCC deposit. When I read the word "hybridise' in those claims I understand it to mean that the hybridisation reaction should be conducted under suitably stringent conditions such that only VEGF-2 polynucleotide sequences would bind either nucleotide sequence shown in the sequence listing (SEQ ID No. 1) or the cDNA deposited in the ATCC deposit identified in the patent specification or fragments thereof.

3.10 A precise match between the sequence of one DNA and the sequence of another complementary DNA is not necessarily required for hybridization to occur and it is possible to vary the exactness of the match required between a DNA and a complementary sequence by changing the conditions under which annealing takes place. Specifically, the more stringent the hybridization conditions, the greater the match required. The hybridization conditions can be made more stringent by raising the annealing temperature and less

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stringent by lowering the annealing temperature. Similarly, lowering the ionic strength of the solution in which hybridization takes place also increases the hybridization stringency. I and I believe any person of ordinary skill in this field would have been aware of how to vary hybridization conditions to specifically isolate a particular protein such as VEGF-2.

3.11 I note that the patent specification in Example 1 refers to conditions which I would expect are sufficiently stringent to prevent cross-hybridisation between polynucleotides that encode VEGF-2 and other polynucleotides. Here, the patent specification teaches two different hybridisation wash conditions: (1) 60 degrees C with 0.5XSCC and 0.1% SDS or (2) 65 degrees C at 0.2xSSSC. Either of these conditions is sufficiently stringent enough to exclude cross hybridisation with unrelated polynucleotides, particularly, VEGF, PIGF, or PDGF. I also note that page 6 lines 2 to 4 in the patent specification states that no nucleotide sequence homology was detected between VEGF-2, VEGF and both forms of PDGF. The low level of identity between VEGF-2 polynucleotides and other VEGF polynucleotides suggests to me that a researcher would actually get good specificity in hybridisation reactions. In particular, given the low level of homology between VEGF-2 polynucleotides and other PDGF/VEGF polynucleotides it is unlikely that cross-hybridisation would occur between VEGF/PDGF polynucleotides and VEGF-2 polynucleotides at these hybridisation conditions.

3.12 I and I believe any other researcher in the molecular biology field would have no difficulty designing suitable hybridisation conditions to specifically isolate VEGF-2 polynucleotides. I do not read the claims that refer to hybridisation to VEGF-2 in the claims to encompass non-VEGF-2 molecules.

Production of functional VEGF-2.

3.13 The patent specification discloses 350 amino acids of the VEGF-2 sequence whereas it has subsequently been determined that VEGF-2 has 419 amino acids. The missing amino acid sequence is now known to contain the signal sequence that directs secretion of VEGF-2 from the cell.

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3.14 The nucleotide sequence shown in Figure 1 actually provides the nucleotide sequence of a further 70 nucleotides of the full VEGF-2 sequence upstream of the putative start codon which encode another 23 amino acids (EATAYASKDLEEQLRSVSSVDEL) in the complete sequence. This means that the nucleotide sequence that encodes 373 amino acids, is given in the HGS application, not 350 amino acids, and therefore only 46 amino acids, not 69 amino acids are missing.

3.15 Since members of the PDGF/VEGF family are growth factors, I would have expected as at March 1994 that any new members would, like VEGF and PDGF, be secreted. In general, most secreted proteins comprise an N-terminal hydrophobic sequence that causes targeting to the endoplasmic reticulum and subsequent secretion (a signal sequence).

3.16 The best way to confirm that a signal sequence functions as such in a biological system is to express the polypeptide containing the signal sequence in a suitable host cell. In the case of VEGF-2 and related molecules this would be a eukaryotic cell.

3.17 The patent specification speculates that the 350 amino acid sequence may contain a signal sequence. If the 350 amino acid VEGF-2 sequence disclosed in the patent specification were expressed in a eukaryotic cell and no secretion observed, I would have looked for reasons why the protein was not secreted in the experimental system. In this respect I was aware by 1994 that it was not uncommon for proteins that were normally secreted to fail to be secreted in an experimental system.

3.18 There may be a number of reasons why a recombinant protein may fail to be secreted. For example, the signal sequence may be silent or inefficient in a particular context. Alternatively, the signal sequence may be incomplete or missing.

3.19 I do not consider the lack of a disclosure of the N-terminal amino acids containing the signal sequence to be a critical omission from the patent specification. The specification describes utilising a heterologous signal sequence capable of directing secretion of the translated protein (see, the patent specification at page 14, lines 6-

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23). Thus, there is a disclosure in the patent specification to fuse the sequence provided to a signal sequence that directs secretion of the protein. By 1994 a number of signal sequences were known and could have been used to direct secretion of a heterologous protein. Even if the disclosed VEGF-2 sequence contained an atypical signal sequence, I would still have linked the sequence disclosed in the patent specification to a strong signal sequence to ensure efficient secretion of the protein. Such research would have been standard practice for me and I believe my colleagues by 1994.

3.20 Consequently, I believe that if the 350 amino acid sequence was expressed but not secreted then I and I believe my colleagues would simply add a signal sequence to ensure efficient secretion. I would do so rather than assume that the 350 amino acid VEGF-2 sequence was not a secreted protein because of the striking conservation of the eight cysteine residues in the middle portion of the molecule, the presence of the 14 amino acid PDGF/VEGF signature motif, and the overall homology with VEGF which was known to be a secreted growth factor.

3.21 The addition of signal sequences was routine by 1994. Indeed, a number of commercial expression vectors were available with signal sequences already present. All a researcher had to do was to clone their sequence downstream of and in frame with the signal sequence. Examples of suitable vectors that were available by 1994 include the Baculovirus Transfer Vectors: pMbac and pPbac described in the 1994 Stratagene Cloning Systems Catalogue on page 45. These vectors have sequences encoding the secretory signal peptides from human placental alkaline phosphatase or mellitin upstream of their cloning sites. Another example is the prokaryotic vector pEZZ 18 (Pharmacia LKB Biotechnology: Molecular and Cell Biology Catalogue 1992, page 5) which contains a protein A signal sequence to allow secretion of the protein into culture medium.

3.22 I am aware that VEGF-2 is proteolytically processed upon secretion from cells *in vivo* to form the naturally occurring ligand for the Flt-4 and the KDR/Flk-1 receptors. I would expect a fusion of the 350 amino acid sequence of VEGF-2 to a signal sequence to be proteolytically processed to produce functional VEGF-2.

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3.23 Thus I consider that the patent specification provides all the necessary information to routinely obtain fully active processed VEGF-2.

3.24 I have been asked to review the first priority document for Helsinki/Ludwig patent specification 711578 (66169/96). I have done this and note that the document shows that the inventors of that patent application also (i) obtained a partial clone encoding a polypeptide of 350 amino acids, (ii) predicted the start methionine to be in exactly the same place as in the HGS patent specification, and (iii) predicted a signal sequence at the start of the 350 amino acid sequence. Further, I am aware that Dr. Alitalo reported these results in Joukov *et al.*, 1996, EMBO Journal 15: 290-298. He subsequently corrected this statement in Joukov *et al.*, 1996, EMBO Journal 15: 1751

3.25 Once the nucleotide sequence of the majority of VEGF-2 had been made available, a skilled person would realise from routine experimentation that the 5' end of the gene was missing from the clone and seek to obtain the additional sequence, using the available sequence to do so. Once it had been established that the clone was a partial clone, it would, be a routine matter to carry out further screening to obtain the full length sequence. Regardless, there is sufficient information in the 350 amino acid form to allow for the expression of a biologically active form of VEGF-2 that is correctly processed.

3.26 I consider the information provided in the patent specification sufficient to allow myself and any other skilled person to practice the invention in relation to expressing functional VEGF-2. In addition, it would have been by 1994 a routine matter firstly to recognise that there was some sequence missing from the VEGF-2 clone and secondly to obtain the additional sequence encoding the N-terminal 46 amino acids of VEGF-2.

Biological activities of VEGF-2 and uses

3.27 The patent specification provides numerous references to examples of properties and activities of VEGF-2 (see, for example, pages 4 and 16 to 17). At the very least, the actual name given to the protein

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would indicate to me that the protein is a vascular endothelial growth factor and therefore would be expected to act as a mitogen towards vascular endothelial cells. It would also direct me to possible means for testing for biological function or activity of that molecule.

3.28 The patent specification discloses that VEGF-2 is involved in angiogenesis (pages 16-17), as a wound healing agent (page 4 third paragraph and page 16 last paragraph that extends onto page 17), to promote endothelialisation (page 4 third paragraph and page 17 fourth paragraph), to treat myocardial infarctions and ischaemia (page 17 third full paragraph) and for *in vitro* culturing of vascular endothelial cells (page 18 first paragraph). The specification also discloses at page 17 last full paragraph extending to page 18 that VEGF-2 is involved in tumour angiogenesis.

3.29 I do not consider the various properties and uses described in the patent specification as exhaustive. The patent specification teaches that VEGF-2 is a member of the PDGF/VEGF family of growth factors. On reading this by 1994 a skilled person would have, as a matter of routine, subject VEGF-2 to a variety of tests and experimental procedures already known in the art for VEGF. For example, the VEGF-2 molecules could be tested using endothelial cell proliferation assays, angiogenesis assays and wound healing assays. All these types of assays were routine by 1994. Given the identification of VEGF-2 as a member of the PDGF/VEGF family of growth factors, I and I believe my colleagues would be looking to see if VEGF-2 actually functioned as a growth factor, which of course it does.

3.30 In addition, page 4 lines 12 to 14 of the patent specification states that the VEGF-2 polypeptides of the invention may be used to isolate receptors of VEGF-2. At page 24 fifth paragraph to page 25 first paragraph the patent specification discloses that VEGF-2 binds to tyrosine kinase receptors on the surface of target cells to activate endothelial cell growth.

3.31 In 1990, a receptor for VEGF was identified, the Flt1 receptor (also known as VEGFR-1) (Shibuya *et al.*, 1990, Oncogene 5: 519-524).

3.32 A further VEGF receptor, the Flk-1/KDR receptor (also known as VEGFR-2) was reported in 1991 (Terman *et al.*, 1992, Biochemical

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and Biophysical Research Communications 187: 1579-1586 and Terman *et al.*, 1991, Oncogene 6: 1677-1683). Both receptors were characterised as members of the receptor tyrosine kinase superfamily. Binding of VEGF to the receptor results in receptor phosphorylation.

3.33 In 1992, (Aprelikova *et al.*, 1992, Cancer Research 52: 746-748) a further orphan receptor, termed "Flt4", was described as a class III receptor tyrosine kinase with significant homology to the Flt1 receptor. The Flt4, Flk1 and Flk1/KDR receptors were referred to as the FLT gene family, tyrosine kinase receptors which bind VEGF molecules, in Kaipainen *et al.*, 1993, Journal of Experimental Medicine 178: 2077-2088 (last sentence of abstract on page 2077).

3.34 Given (i) that the patent specification states that the VEGF-2 protein may be used to isolate receptors for VEGF-2, and (ii) that the patent specification states that VEGF-2 may bind to tyrosine kinase receptors and (iii) the availability of three members of a family of receptors, one of which had no known ligand while the other two were known to bind VEGF, I would expect a skilled person to consider whether the VEGF-2 protein could bind to any one of Flt1, Flk1/KDR and Flt4.

3.35 Since the patent specification teaches a new member of the PDGF/VEGF family with no known receptor and the prior art teaches an orphan receptor in the same family as the receptor for VEGF with no known ligand, I and I believe others in my field would consider it a matter of routine to test VEGF-2 for binding to the Flt-4 receptor, the Flt1 receptor and the Flk-1/KDR receptor.

3.36 I consider the patent specification to teach a number of properties of and uses for VEGF-2. Furthermore, I and I believe others of ordinary skill in my field would be able to use the information provided in the patent specification to produce VEGF-2 protein and confirm that VEGF-2 does indeed have the properties discussed above, as well as obtaining further information about VEGF-2, using a variety of routine techniques already in use by researchers by 1994 to study growth factors such as VEGF.

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Uses of fragments of VEGF-2 and VEGF-2 antibodies

3.37 Associate Professor Rogers has commented that fragments of polypeptides have no practical utility whatsoever (see for example paragraph 4.10.2, Associate Professor Rogers' Statutory Declaration). Clearly this is not the case. As asserted in the patent specification, fragments of polypeptides can be used to make antibodies, which are useful both experimentally and therapeutically. In addition, active fragments of polypeptides are often used in therapy as antagonists since they can compete with the full length version but may lack full biological activity. Further, such polypeptide fragments could also be used as agonists that might mimic some of the biological activities of the full-length protein. Although the skilled person would be aware of this in any case, I note that the specification discloses the following:

3.37.1 Page 22 last full paragraph that extends to page 23 teaches that fragments may be used to make antibodies to the polypeptide [VEGF-2]. Pages 22-23 of the patent specification describe suitable techniques for producing antibodies that bind to VEGF-2

3.37.2 Page 23 last paragraph to page 24 second paragraph teaches that the antibodies may be used to block the activity of VEGF-2, in a similar manner as has been demonstrated previously for VEGF.

3.37.3 Page 24 first paragraph teaches that VEGF-2 antibodies may be used to measure elevated levels of VEGF-2 in individuals. It is in fact common for cancerous conditions to be associated with increased levels of growth factors.

3.37.4 Further, the patent specification clearly demonstrates that VEGF-2 is expressed at higher levels in cell lines derived from malignant tumours (page 18 lines 1 to 5).

3.37.5 Page 24 fifth paragraph teaches that truncated versions of VEGF-2 that fail to activate endothelial cell growth may be used as an antagonist.

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4. Comments on Associate Professor Rogers' Statutory Declaration

4.1 Associate Professor Rogers has raised a number of points in his statutory declaration. However, many of the points are repetitive and so I have sought to deal with them in a more structured manner. Consequently, a lack of reference to a specific item in Associate Professor Rogers' statutory declaration should not be taken as an admission that I necessarily agree with his comments.

The meaning of VEGF-2

4.2 One issue that Associate Professor Rogers comments on is the meaning of "VEGF-2 fragments, analogues and derivatives". I set out in paragraphs 3.4 to 3.8 my understanding of these terms from my reading of the patent specification. I would reiterate that, by contrast to Associate Professor Rogers' comments in paragraph 2.2 and 2.3 of his statutory declaration, I do not consider VEGF-2 fragments, analogues and derivatives to encompass PDGF, VEGF or PIGF polypeptides or polynucleotides. Specifically, I do not consider PDGF, VEGF or PIGF to be analogues or derivatives of VEGF-2 in the same way that I do not believe that a skilled person would consider VEGF to be a fragment analogue or derivative of PDGF. They are different molecules.

VEGF-2 activity

4.3 Associate Professor Rogers asserts in his declaration that the patent specification has not taught a unique defining activity that can serve to discriminate PDGF/VEGF from VEGF-2. However, I do not see how this is relevant, nor do I agree that it is necessary to do so. Once a substantial portion of the primary amino acid sequence is available, this provides the essential defining characteristic.

4.4 Associate Professor Rogers also makes the general point that he considers the patent specification does not provide a demonstration or guidance as to VEGF-2 biological activity (paragraph 2.3.2, paragraph 4.6). However, I consider that the patent specification provides a variety of information and guidance about the biological activity of VEGF-2 (e.g. on page 4 and pages 16 to 18 of the patent specification). I refer to and repeat my detailed comments in

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paragraphs 3.27 to 3.39 and 3.37. Furthermore, I, and I believe a skilled person when presented with the information in the patent specification, would be able to obtain confirmation of these biological activities using techniques that were routinely available and used by 1994, such as angiogenesis assays and endothelial cell proliferation assays referred to in paragraph 3.29 above.

4.5 Associate Professor Rogers states in paragraph 4.6.1 of his statutory declaration:

> "...that the specification fails to demonstrate any VEGF2 biological activity is especially significant in that the biological activities of VEGF2 (if any) is a concept analogous to utility: if one does not know the activities of VEGF2, one would not know how to use VEGF2 (except as a curiosity for further research)"

4.6 In Example 1 in the patent specification the inventors demonstrate that VEGF-2 is over expressed *in vivo* in a number of malignant breast tumour cell lines. This result is, I believe, indicative of VEGF-2 biological activity. It suggests to me that VEGF-2 plays a role in tumour development possibly by promoting new blood vessel growth similar to VEGF. Additionally, it is apparent to me from reading the patent specification as I believe it would be to others in my field that VEGF-2 is a growth factor that is related to VEGF and is likely to play a role in the regulation of endothelial cell mitogenesis.

4.7 I am unaware of any evidence that suggests that VEGF-2 does not possess the activities identified in the patent specification. Further, by 1994 it would have been routine for a skilled person to confirm that these proposed activities were correct. For example, testing polypeptides for effects on the growth of cells such as endothelial cells (as set forth on page 18 lines 6 to 8 of the specification) was routine by 1994. It was also routine for a skilled person to test for wound healing using a standard punch biopsy.

4.8 I also add that neither I nor any of my colleagues would expect that the initial characterisation of a gene should provide a full description, supported by detailed experimental proof, of every property and function that the encoded protein possesses.

4.9 Further, once the VEGF-2 sequence of the patent specification was identified, I do not believe that an ordinarily skilled person would

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have had any difficulty by 1994 in producing analogues, fragments and derivatives of VEGF-2. The same routine ease would have also applied to the testing of VEGF-2 analogues, fragments and derivatives for VEGF-2 activity.

4.10 In response to paragraph 5.5 (5.5.1) of Associate Professor Rogers' declaration, I consider the process of determining which parts of a polypeptide molecule are required for activity to have been entirely routine by 1994. Suitable strategies would have included deletion mutagenesis or site-directed mutagenesis, which involves deleting or replacing regions of the molecule and determining the effect on protein function. Additionally, the specification highlights the 8 cysteines and the 14 amino acid signature motif thought to be involved in VEGF-2 function.

Hybridising and immunologically cross-reactive polypeptides

4.11 In paragraph 2.4 of his statutory declaration, Associate Professor Rogers' comments that the limitation "hybridising to" does not serve to distinguish the claimed polynucleotides from polynucleotides encoding VEGF, PlGF and PDGF. However, as stated in paragraphs 3.9 to 3.12 above, I consider that it is unlikely that there would be any cross-hybridisation between VEGF-2 polynucleotides and polynucleotides encoding VEGF, PlGF and PDGF under suitable hybridisation conditions such as those provided in Example 1, particularly given the lack of detectable homology between VEGF-2 and other members of the PDGF/EVGF family (page 6 lines 2 to 5 of the specification) at the polynucleotide level.

4.12 Further, in response to Associate Professor Rogers comments in paragraphs 2.7.5 and 4.10.5, I do not consider it likely that a polynucleotide hybridising under these conditions and cross-reactive with an antibody that binds to VEGF-2 would encode anything other than a VEGF-2 molecule or a immunologically reactive fragment of VEGF-2. The dual test provided by claims 16 to 27 and 40 to 50 (when read in conjunction with the specification) is, I believe, actually much more stringent than Associate Professor Rogers has alleged. I am not aware of any polypeptides/polynucleotides that would satisfy the criteria of claims 16 to 18 and 40 to 45 but which are not VEGF-2 or an immunologically reactive fragment of VEGF-2.

4.13 Similar comments apply to Associate Professor Rogers' statements in paragraph 4.10.1 where he draws attention to the lack of an explicit reference in claims 16 to 18 and 40 to 45 to "activity". However, the polypeptides encoded by the polynucleotides referred to in these claims are all immunologically cross-reactive with an antibody that binds to a VEGF-2 polypeptide, and are therefore biologically active fragments. Consequently, I consider these claims to require implicitly the polypeptides to have VEGF-2 activity.

4.14 My comments in paragraphs 3.3 and 3.8 above are also relevant to paragraphs 5.7.2 to 5.7.3 where Associate Professor Rogers has referred to the "enormous scope of these claims". For the reasons set out in paragraphs 3.2 to 3.8, I believe that the claims are limited to VEGF-2 polypeptides or a immunologically reactive fragment of VEGF-2.

4.15 Associate Professor Rogers comments in paragraph 5.7.1 that the language used in claims 16 to 18 and 40 to 45 that refers to "a polypeptide which binds to an antibody capable of binding to VEGF-2" is not found in the specification. However, page 23 lines 5 to 14 refer to polypeptides that are bound by antibodies generated against polypeptides of the invention. Since the polypeptides of the invention are VEGF-2 polypeptides, I interpret this section as corresponding to a disclosure of a polypeptide that is bound by an antibody to VEGF-2.

Production of antibodies

4.16 Associate Professor Rogers also comments in paragraph 4.10.2 that no antibodies are exemplified in the specification. In my opinion, given the disclosure of the VEGF-2 sequence in patent specification, biological scientists, from Ph.D. students to heads of Department, would have had no difficulty by 1994 in obtaining antibodies to VEGF-2 using routine techniques such as immunisation of rabbits with purified VEGF-2 protein. They would not need a demonstration in the patent specification to enable them to do this. Nonetheless, page 23 second and third paragraphs of the patent specification describes suitable techniques for producing antibodies.

NA

Disclosure of VEGF-2 sequence

4.17 In paragraph 4.4, Associate Professor Rogers comments that the patent specification fails to disclose the complete amino acid and polynucleotide sequence of VEGF-2. In particular, Associate Professor Rogers comments in paragraph 4.11.1.2 that the patent specification fails to teach the N-terminal 69 amino acids of the full length molecule which contains the signal sequence "which is crucial for directing secretion of VEGF2 in cells". I should note that whilst a signal sequence is necessary for secretion, there is no requirement to use the signal sequence that is normally found at the N-terminus of the newly expressed VEGF-2 molecule.

4.18 The thrust of Associate Professor Rogers' arguments appears to me to be that HGS should not be entitled to claim the full length VEGF-2 molecule because only polynucleotides encoding 350 amino acids (or 373 amino acids in actual fact) are disclosed in the patent specification. However, I consider that the information provided in the patent specification would allow the skilled person to obtain the complete sequence without any difficulty, as discussed in 3.13 to 3.26.

4.19 In this regard, I also consider that the information provided in the patent specification would allow the skilled person to obtain VEGF-2 nucleotide sequences from other species (so-called orthologues) by routine techniques. Consequently, I disagree with Associate Professor Rogers' comments in paragraph 5.6 that the specification provides no basis for non-human forms of VEGF-2.

4.20 Associate Professor Rogers also appears to consider that the specification does not teach a VEGF-2 molecule that possesses biological activity because the sequence of the N-terminal 69 amino acids found in the full length molecule is not provided in the patent specification (see for example paragraph 7.6). I have set out my comments at length in paragraphs 3.13 to 3.26 as to why I believe that the specification teaches the skilled person how to obtain biologically active VEGF-2. In particular, I believe that faced with any difficulties in obtaining secretion of the 350 amino acid VEGF-2 polypeptide disclosed in the specification, I and I believe others of ordinary skill in the PDGF/VEGF field, would simply add/substitute a

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signal sequence and the resulting protein would be properly processed by a suitable host cell to produce active VEGF-2. As demonstrated by Dr Alitalo's own work, fragments smaller than the 350 amino acid sequence, which retain the signature motif and cysteine residues, contain sufficient information to be processed to a biologically active form. (See, U.S. Patent No. 6,130,071, issued October 10, 2000, to Alitalo et al., column 47 line 57 to column 48 line 2)

Examples in the Patent Specification

4.21 Associate Professor Rogers has made some comments in paragraph 4.13 on a number of apparent errors.

4.22 In paragraph 4.13.1. Associate Professor Rogers comments that the size of the mRNA referred to in Example 1 (1.6 kD) is inconsistent with subsequent Northern hybridisation studies. The 1.6 kD actually refers to a Northern blot shown as Figure 4 where a number of breast cancer cell lines were tested. Two strong bands are seen in lanes 4 and 6 of the autoradiograph shown in Figure 4. These two lanes correspond to total RNA from two breast tumour cell lines. The size markers on the right hand side appear to me to correspond to 18S and 28S ribosomal RNA, which have molecular weights of about 2.2 and 4.5 kb, respectively. Thus the strong bands in lanes 4 and 6 are clearly less than 2.2 kb in size and 1.6 kb would not be an unreasonable interpretation. (As a minor point the reference to "kD' in the specification and not "kb" is obviously an error and one I would expect to be recognised by one of ordinary skill. Such a person would appreciate that "kb" was the intended term since the size of RNA on a Northern blot is measured in kilobases not kiloDaltons).

4.23 Interestingly, a corresponding band is not seen in the lane corresponding to normal breast cells or in most of the other breast tumour cell lines. Consequently, it appears likely to me that the results shown in Figure 4 show that a 1.6 kb RNA that hybridises to a VEGF-2 probe is highly over-expressed in two breast tumour cell lines.

4.24 Figure 5, by contrast, shows the results obtained with a range of normal cells from different tissues i.e. Figures 4 and 5 show the

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results of different experiments. The two bands seen in lanes 1 to 10 are indicated on Figure 5 to be 2.2 kb and 1.3 kb in size. However, the 1.3 kb band has a greater molecular mass than the 1.4 kb molecular weight marker on the left hand side of the Northern blot. I would judge the two bands to be about 2.4/2.3 kb and 1.6 to 1.8 kb. Thus at least the major band is reasonably consistent with subsequent results.

4.25 Although there are two minor errors with respect to "kD' instead of "kb" and a mislabelled band in Figure 5, I had no difficulty in understanding the results presented.

4.26 In paragraph 4.13.3 of his statutory declaration, Associate Professor Rogers has criticised the expression studies conducted in Example 2 of the patent specification. Specifically, Associate Professor Rogers has commented that the lack of a sequence for the F5 primer means that it is not clear how the third primer pair can produce a PCR product that encodes the full polypeptide of VEGF-2. I disagree. Since the sequence of the complete VEGF-2 cDNA insert is given in Figure 1 of the specification, it would be a simple matter for the skilled person to design a suitable primer that hybridises to a region between the VEGF-2 stop codon and the 3' end of the insert. Indeed, it would be a simple matter for the skilled person to produce a variety of different length PCR products containing all or some of the VEGF-2 cDNA insert with the information provided in Figure 1.

4.27 With regard to the second primer pair that produces a PCR product encoding a polypeptide missing 36 amino acids from the C-terminus of VEGF-2, there does appear to be an error in Example 2. The specification states that the F4 primer is "from bp 1259 to 1239, about 169 bp away from the 3' end of the stop codon and about 266 bp before the last nucleotide of the cDNA." (page 28 lines 22 to 26). When compared with the sequence of Figure 1, this is clearly within the 3' UTR. Consequently, the sequence given for the F4 primer when combined with an M13 reverse primer would give the full VEGF-2 product. It would therefore appear to me that the F4 primer sequence is actually the F5 primer sequence and the F4 primer is missing.

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4.28 Since Example 2 teaches that the second primer pair amplifies a PCR product encoding a polypeptide missing 36 amino acids from the C-terminus of VEGF-2, and the nucleotide sequence of VEGF-2 is given in Figure 1, it would have been possible for me to design by 1994 a suitable primer using the Figure 1 information. I have in fact designed such a primer as an example, using only the information in the specification. The primer sequence is: 5' ACAGCTGCATGTTT GGTGG 3'. Therefore the fact that the primer is missing is not in my opinion a problem.

4.29 While there are some errors in Example 2, it clearly shows that the VEGF-2 cDNA can be expressed *in vitro* to produce a polypeptide product. In any case, with the information provided in the specification, in particular Figure 1, the skilled person could easily carry out similar experiments to those shown in Example 2 without any specific guidance as to the primer sequences etc., and obtain similar results.

5. Comments on Dr Alitalo's Statutory Declaration

5.1 Dr Alitalo describes in his statutory declaration some expression studies using expression plasmids containing a construct encoding amino acids 70 to 419 of full length VEGF-2 (i.e. amino acids 1 to 350 of VEGF-2 as described in the patent specification) or a construct encoding the complete 419 amino acid sequence of VEGF-C.

5.2 The plasmids were transiently transfected into a mammalian cell line (293T cells). Both cell lysates and the culture medium were tested for the presence of newly synthesised VEGF-2/VEGF-C proteins (which are radioactively labelled due to the inclusion of radioactively labelled methionine in the culture medium). The results are shown in Exhibit 3.

5.3 VEGF-2/VEGF-C were partially purified from the cell medium and cell lysates using an immunoprecipitation procedure. This involves using antibodies to bind to the VEGF-2/VEGF-C. However, as discussed in paragraph 6.5 of Dr. Alitalo's statutory declaration, the antibody used to immunoprecipitate VEGF-2(70-419) was a monoclonal antibody that recognises hemagglutinin (HA), a peptide tag which was fused to the C-terminus of VEGF-2. By contrast, the antibody used to

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immunoprecipitate VEGF-C (1-419) was a polyclonal antibody, which recognises residues 31 to 51 of the 350 amino acid VEGF-2 polypeptide.

5.4 In Alitalo's own published work, he has reported the inability to isolate VEGF-C using an antiserum against the C-terminal amino acid residues 372-394 or by using a tag attached to the C-terminus (Joukov et al., 1997, EMBO J. 16: 3898, at 3900). Thus, by Alitalo's own admission an antibody to a tag at the C-terminus of VEGF-2 will not successfully immunoprecipitate the protein.

5.5 HGS scientists have reported the successful isolation of a modified VEGF-2 protein containing an HA tag at its carboxy terminus using a monoclonal anti-HA antibody. (See, HGS Australian Patent No. 714484 and Hu JS et al (1997) FASEB J May;11(6):498-504). These studies were conducted in COS cells, whereas the experiments set forth in Dr. Alitalo's declaration were conducted in 293T cells. The significance of the different cell types used is provided by Dr. Alitalo's own publications (Joukov et al., 1997, EMBO J 16: 3898-3911). This publication describes the proteolytic processing of VEGF-2 when expressed by a number of different cells lines, including COS cells, PC-3 cells, HT 1080 cells, and 293 EBNA cells. The results of this comparison, as reported by Dr. Alitalo was that "[t]he proteolytic processing of the VEGF-C precursor in COS cells was less efficient when compared with other cell types." (See Joukov, at page 3901, second column). Thus, as the VEGF-C precursor is processed less efficiently in COS cells, one would also expect that the cleavage of the HA tag from the carboxy terminus would also be less efficient in COS cells, as compared to 293T cells. Hence, the lack of efficient cleavage of the HA tag from the carboxy terminus of the protein when expressed in COS cells may account for HGS' successful isolation of VEGF-2 protein containing an HA tag at its carboxy terminus.

5.6 It is not clear to me why different antibodies were used, particularly since the VEGF-C antibody would have recognised both VEGF-C and VEGF-2. Importantly, however, the use of two different antibodies introduces three major flaws into the experimental design, which prevent conclusions from the ... drawn from the data obtained and shown in Exhibit 3.

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5.7 First, even different antibodies that bind to the same polypeptide tend to have different affinities for the region of the polypeptide to which they bind. This means in practical terms that for a given amount of the protein, the amount detected or bound by each antibody often differs. Thus, the results obtained with two different antibodies cannot be compared quantitatively due to the lack of any controls. In this case the antibodies are even against different proteins (VEGF-C and HA). Consequently, it is not possible to make any kind of quantitative comparison between the results for VEGF-C and VEGF-2 shown in Exhibit C since the differing efficiency with which the different antibodies bind to their target polypeptides will affect the proportion of protein that they are able to bind in the culture medium and cell lysates. Furthermore, one antibody is monoclonal and one is polyclonal and so a comparison of the results is even more difficult to make. Also, no indication is given as to the amount of polyclonal antibody used.

5.8 Second, the aim of the experiment is to determine whether VEGF-2(HGS) is processed and secreted by a mammalian cell. However, whereas the antibody used to detect VEGF-C binds an epitope which is present in the fully processed VEGF-C molecule, the antibody used to detect VEGF-2 binds to a heterologous fusion protein tag (HA) located at the C-terminus of the unprocessed molecule. If VEGF-2 is proteolytically processed to form mature VEGF-2/VEGF-C, the HA tag will be cleaved from the mature part of the molecule. The mature VEGF-2 molecule, no longer being linked to the HA tag, would not be immunoprecipitated and would not therefore appear in either the immunoprecipitated cell medium or immunoprecipitated in the cell lysate, having being discarded when the protein A-sepharose is washed. Thus, in the case of VEGF-C expression, the experimental design allows for the detection of both unprocessed and mature VEGF-C whereas in the case of VEGF-2 expression, only the unprocessed form can be detected and not the mature form. Furthermore, Alitalo's own work indicates that an antibody to a C-terminal tag may not be effective in isolating VEGF-C.

5.9 It is not therefore possible to draw any conclusions about the relative efficiency of secretion of VEGF-C and VEGF-2 (70 to 419) from these data - the same antibody should have been used in both cases.

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5.10 Third, no controls have been carried out to determine the transfection efficiency of the plasmids used - making any comparisons even more difficult.

5.11 Dr Alitalo also states in paragraph 7.2 that

> "it is readily apparent from the autoradiogram [Exhibit 3] that the expression level of VEGF-C is much higher than that of VEGF2(HGS)".

5.12 Further, Dr Alitalo states in paragraph 8.3 that

> "the fact that VEGF-C expression observable in cell lysates of VEGF-C transfected cells is much higher than VEGF2(HGS) expression observable in VEGF2(HGS) transfected cells suggests that VEGF2(HGS) is inefficiently translated and/or that the intracellular turnover rate of VEGF2(HGS) is much faster than that of VEGF-C. In other words, the cells may be recognizing VEGF2(HGS) as an aberrant protein and rapidly degrading it".

5.13 Neither of these conclusions are supported by the flawed data shown in Exhibit 3. As discussed in paragraph 5.3 to 5.6 above, it is not possible to draw any conclusions from these data based on quantitative comparisons. The conclusions set out in paragraphs 7.2 and 8.3 are mere speculation that is unsupported by the results obtained.

AND I make this solemn declaration by virtue of the Statutory Declarations Act, 1959 and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DATED this day 8th of December 2000.

DECLARED at: Brisbane, Queensland)
BEFORE me: .....)

N. Hayward
.....
Nicholas Kim Hayward

.....
Patent Attorney

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COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure NKH-1** referred to in my Statutory Declaration made this 8th day of December 2000.

N. Hayward

Nicholas Kim Hayward

WITNESS: _____

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

CURRICULUM VITAE

NAME: Nicholas Kim Hayward

WORK ADDRESS: Human Genetics Laboratory
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TERTIARY QUALIFICATIONS:

1981-1983 Doctor of Philosophy, Biochemistry Department, University of Queensland - project titled "Phenacetin metabolites and DNA damage". Conferred 7/7/83.

1980 Master of Science, Qualifying, Biochemistry Dept. University of Queensland (UQ) - passed at the level of first class honours.

1977-1979 Bachelor of Science, University of Queensland - majoring in Biochemistry and Microbiology.

POSITIONS HELD:

Current:

- NH&MRC Senior Research Fellow.
- Head, Human Genetics Laboratory, Queensland Institute of Medical Research (QIMR).
- Chair, Cancer and Cell Biology Division, QIMR
- Chair, Joint Experimental Oncology Programme of the QIMR, the University of Queensland and the Queensland Cancer Fund.
- Conjoint lecturer - Department of Pathology, University of Queensland.
- Senior Research Fellow, Queensland Centre for Schizophrenia Research, Wolston Park Hospital, Wacol, QLD 4076.
- Faculty Representative, QIMR Staff Association
- Faculty Representative, QIMR Scientific Advisory Committee
- Faculty Representative, QIMR Joint Consultative Committee

Previous:

1994-1997 NH&MRC Research Fellow
1991-1993 NH&MRC R. D. Wright Fellow
1983-1990 NH&MRC research officer/senior research officer - QIMR.
1982 Group 1 tutor: Medicine and Pharmacy students - University of Queensland.
1982 Private tutor: Medical students - University of Queensland.
1980-1981 Casual tutor: Science, Dentistry and Pharmacy students - University of Queensland.

AWARDS:

NH&MRC Senior Research Fellowship 1998-2002.

NH&MRC Research Fellowship 1994-1997.

Queensland Cancer Fund Travel Fellowship 1994.

NH&MRC R. D. Wright Fellowship 1991-1993.

Beckman-QIMR Young Scientist Award 1991.

Queensland Cancer Fund Travel Fellowship 1990.

Commonwealth Postgraduate PhD Scholarship 1981-1983.

PATENTS:

Patent - through the Council of the Queensland Institute of Medical Research and AMRAD Corporation Ltd. for the invention titled: "A novel growth factor and a genetic sequence encoding same".
Patent application number: PCT/AU96/00094.
* This patent covers the VEGFB gene and its therapeutic use.

Patent - through The Council of the Queensland Institute of Medical Research and AMRAD Corporation Ltd. for the invention titled: "A novel gene and uses therefor".
Patent application number: PCT/AU98/00380.
* This patent covers the RASGRP2 (MCG7) gene and its therapeutic use.

Provisional patent - through The Council of the Queensland Institute of Medical Research and AMRAD Corporation Ltd. for the invention titled: "A method of treatment and prophylaxis".
Patent application number PQ5921/00 (filed 29/02/00).
* This patent covers the therapeutic use of the VEGFB gene or related agonists or antagonists in treatment of rheumatoid arthritis.

SOCIETY MEMBERSHIPS:

Human Genetics Society of Australia
National Association of Research Fellows (NH&MRC)

COMMITTEE/PANEL MEMBERSHIPS:

Grant review panels

Cancer Research Grant Discipline Panel (Biochemistry, Molecular Biology and Genetics) – 2000

QCF Grant Review Panel 1991-current

Conference committees

Clinical Oncological Society of Australia 2001, Brisbane - Programme Committee 1999-current

Clinical Oncological Society of Australia 1996, Brisbane - Programme Committee 1995-1996

Australia and New Zealand Environmental Mutagens Society 1996, Mount Buller - Programme Committee 1995-1996

Queensland Cancer Fund International Conference on Melanoma 1994, Brisbane - Steering Committee 1992-1994

Institutional Committees

QIMR Comprehensive Cancer Research Centre Opening Scientific Conference 2001, Brisbane - Steering Committee 2000 - current

QIMR Scientific Advisory Committee 1999-current

QIMR Staff Association 1999-current

QIMR Comprehensive Cancer Research Centre User/Advisory Groups (Laboratory, Waste, Freezers) 1999-2000

QIMR Joint Consultative Committee, Faculty Representative 1998-current

QIMR Student Committee 1992-1994

QIMR Seminar Committee 1989

QIMR Library Committee 1986-2000

QIMR Conversazione Committee 1985-1992

EDITORIAL DUTIES:

Associate editor for *Clinical Cancer Research*

Editorial committee member for *Disease Markers*

JOURNAL REVIEWER FOR:

British Journal of Cancer
Cancer
Cancer Research
Clinical Cancer Research
Cell Stress and Chaperones
European Journal of Cancer
Genes Chromosomes and Cancer
Human Mutation
International Journal of Cancer
Journal of Gastroenterology and Hepatology
Journal of Investigative Dermatology
Journal of Medical Genetics
Oncogene

GRANT REVIEWER FOR:

Anticancer Council of Victoria
Australian Research Council
Clive and Vera Ramaciotti Foundations
Commonwealth Department of Veterans Affairs
Diabetes Australia Research Trust
Government Employees Medical Research Fund
Health Research Council of New Zealand
Leo and Jenny Foundation
National Health and Medical Research Council of Australia
National Institute of Forensic Science
New South Wales Cancer Council
Raine Foundation
United States-Israel Binational Science Foundation
University of Queensland Research Services Special Grants Scheme
University of Sydney Cancer Research Fund

INVITED CONFERENCE PRESENTATIONS:

5th World Conference on Melanoma – Venice – 1/3/01

Prevention of Melanoma and Skin Cancer Conference – Brisbane 5/12/00

Australian Society of Medical Research 39th Annual Meeting – Melbourne 28/11/00

Speaker and session chairman, American Association of Cancer Research – San Francisco 1/4/00

3rd Peter MacCallum Symposium – Initiation and Progression of Cancer – Melbourne 8/11/99

Combio Conference – Broadbeach 30/9/99

Lorne Cancer Conference - Lorne 1998

Royal Brisbane Hospital Health Care Symposium - Brisbane 1997

4th World Conference on Melanoma - Sydney 1997

Royal Australasian College of Surgeons Annual Scientific Congress - Brisbane 1997

The Mount Buller International Conferences on Environmental Radiation - The Environmental and Health Effects of Solar Radiation - Mount Buller 1996

23rd Clinical Oncological Society of Australia Conference - Brisbane 1996

7th Frank and Bobbie Fenner Conference in Medical Research - Molecular Mechanisms in Cancer- Canberra 1996

Australian Workshop on Cancer Gene Analysis and Mutation Detection - Noosa 1996

Italy-Australia Scientific Weeks 1996: Oncology - Brisbane 1996

10th Annual Oncology Nursing Symposium - Toowoomba 1994

Greenslopes Hospital Week Symposium - Brisbane 1993

Speaker and session chairman at the International Conference on Mutagens and Modulators of Gene Expression - Brisbane 1993

Lorne Cancer Conference - Lorne 1993

Speaker and session chairman at the Human Genetics Society of Australia meeting - Brisbane 1992

Melanoma Symposium - Brisbane 1988

Molecular Biology Symposium - Brisbane 1986

INVITED WORKSHOP PRESENTATIONS:

NCI Mouse Models of Human Cancer Consortium – Cutaneous Oncology – Puerto Rico 31/1/01

International Linkage Consortium for Familial Melanoma – Philadelphia 10/00

International Linkage Consortium for Familial Melanoma – Bethesda 6/00

International Linkage Consortium for Familial Melanoma - Bethesda 6/99

International Linkage Consortium for Familial Melanoma – San Francisco 10/99

International Linkage Consortium for Familial Melanoma - Bethesda 6/98

International Linkage Consortium for Familial Melanoma - Baltimore 10/97

Australian Cancer Genetics Consensus Meeting - Melbourne 1996

Session Chairman at the Fifth International Workshop on Multiple Endocrine Neoplasia - Stockholm 1994

American Society for Human Genetics 42nd Annual Meeting - Linkage analysis of familial melanoma - San Francisco 1992

International Association for the Study of the Liver Satellite Meeting - Molecular genetics of hepatocellular carcinoma - Brisbane 1990

OTHER CONFERENCE PRESENTATIONS:

In addition to the invited presentations listed above I also presented work at the following conferences:

1997	6th International Workshop on Multiple Endocrine Neoplasia, Noordwijkerhout (talk)
1995	45th American Society for Human Genetics Annual Meeting, Minneapolis (poster)
1994	44th American Society for Human Genetics Annual Meeting, Montreal (poster)
1990	41st American Society for Human Genetics Annual Meeting, Cincinnati (poster)
1990	12th Conference on Organization and Expression of the Genome, Lorne (poster)
1990	1st International Conference on DNA Fingerprinting, Berne (poster)
1990	International Association of the Study of the Liver Meeting, Broadbeach (poster)
1988	XII Human Genetics Society of Australasia meeting, Brisbane (poster)
1988	IV International Congress of Cell Biology, Montreal (poster)
1988	XVI International Congress of Genetics, Toronto (poster)
1987	31st Australian Biochemical Society meeting, Perth (talk plus poster)
1985	10th Australian Institute of Nuclear Science and Engineering Radiation Biology Conference, Sydney (talk)
1981	Australian Society for Medical Research, Brisbane (talk)

INVITED LECTURES AT TERTIARY INSTITUTIONS:

University of Queensland, Graduate Medical Course - 1997, 1998
Queensland University of Technology, Faculty of Life Sciences - 1991, 1992, 1993, 1995, 1996
University of Queensland, Biochemistry Department - 1984

INVITED SEMINARS:

National Institute for the Study and Cure of Cancers – Milan, Italy – 5/7/99
Children's Medical Research Institute, Sydney - 1997
Mater Misericordiae Hospital, Brisbane - 1997
Oncology Research Centre, Prince of Wales Hospital, Sydney - 1997
Wesley Hospital, Brisbane - 1996
Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, USA - 1995
AMRAD Corporation, Melbourne - 1995
Queensland Cancer Fund Oncology Nurses Group, Bundaberg - 1994
Centre for Molecular and Cellular Biology, University of Queensland - 1994
Mount Vernon Hospital, England - 1994
Ludwig Institute, Melbourne - 1993
Garvan Institute, Sydney - 1992
Westmead Hospital, Sydney - 1992
Biochemistry Department, University of Queensland - 1984
Royal Brisbane Hospital - 1980

STUDENTS:

I was the principal supervisor of the following graduates:

Sean Grimmond - PhD (University of Queensland) - 27/7/94 (subsequently obtained a C.J. Martin fellowship from the NHMRC)

Derek Kennedy - MMedSc (University of Queensland) - 17/2/95 (subsequently obtained a PhD from the University of Queensland)

Graeme Walker - PhD (Griffith University) - 23/2/95 (subsequently obtained a C.J. Martin fellowship from the NHMRC)

Lee Bergman - BSc(hons) (University of Queensland) - 11/97 - pass level = first class.

Daphne Macaranas - BSc(hons) (University of Queensland) - 11/97 - pass level = class 2A.

Derek Nancarrow - PhD (University of Queensland) – 30/7/99.

Pamela Pollock - PhD (University of Queensland) – 6/3/00 (subsequently obtained a C.J. Martin fellowship from the NHMRC)

I was a cosupervisor of the following graduates:

Teh Bin Tean - BMedSc (University of Tasmania) – 11/89 - (subsequently obtained a PhD from the Karolinska Institute, Stockholm, Sweden)

Hii Su Ing - BSc(hons) (Griffith University) – 11/93 - pass level = class 2A

Amanda Milligan - BSc(hons) (University of Queensland) - 11/95 - pass level = first class

Colin De Souza - PhD (University of Queensland) - 20/1/97

Ian Tonks - PhD (University of Queensland) - 24/3/97

Louise Sparrow - PhD (University of Western Australia) - 1998

Mark Romero – BSc (hons) (Griffith University) – 18/12/98 - pass level = first class

I am the principal supervisor of the following students:

Clare Boothroyd - MMedSc (University of Queensland) - submitted.

Christine Stewart - PhD (Queensland University of Technology) - currently in fourth year.

Lee Bergman - PhD (University of Queensland) - currently in third year.

I am a cosupervisor of the following students;

Jennifer Taylor – PhD (University of Queensland) - currently in third year.

Ellen Zevering - PhD (University of Queensland) - currently writing up/deferred.

Andrew Burgess - PhD (University of Queensland) - currently in first year.

In addition, I was a project supervisor for BC351 - University of Queensland Third Year Medical Molecular Genetics Course, and am a mentor for Literature Review topics within the Graduate Medical Course of the University of Queensland.

THESIS EXAMINER FOR:

Meryta May - BMedSc (University of Queensland) - 1993

Corinne Gustafson - PhD (University of Queensland) - 1996

David Walker - PhD (University of Queensland) – 1997/1998

MAJOR RESEARCH INTERESTS:

- Molecular and cellular biology of multiple endocrine neoplasia type 1.
- Linkage analysis, positional cloning and candidate gene analysis to determine novel familial melanoma genes (Collaboration with J. Trent and M. Brownstein).
- Use of cDNA expression microarrays to study cancer (Collaboration with J. Trent and S. Grimmond).

- Analysis of germline and somatic mutations in melanoma patients.
- Linkage and candidate gene analysis to determine the chromosomal position of genes involved in schizophrenia (Collaboration with B. Mowry and D. Nancarrow).
- Linkage and candidate gene analysis to determine the chromosomal position of genes involved in the development of moles (Collaboration with N. Martin).
- Generation of murine models of melanoma (Collaboration with G. Kay and G. Walker).
- Generation of murine models of multiple endocrine neoplasia type 1 (Collaboration with G. Kay).
- Study of the role of *Vegfb* in mouse development, pathology and neoplasia (Collaboration with G. Kay and A. Mould).

MAJOR SCIENTIFIC ACHIEVEMENTS:

- cloned and characterized the vascular endothelial growth factor B gene (*VEGFB*).
- cloned and characterized the *RASGRP2* gene – encoding a novel guanine nucleotide exchange factor.
- contributed to the independent isolation and characterization of the multiple endocrine neoplasia type 1 (*MEN1*) gene.
- contributed to the identification of the *CDK4* gene as a melanoma susceptibility locus.
- contributed to the generation of the first knockout mouse from QIMR (*Vegfb*).
- contributed to the generation of the first transgenic mouse from QIMR (antisense *Sod2*).
- first and only one to have carried out a linkage search covering the whole genome to determine the location of melanoma susceptibility genes.
- first to confirm the location of a melanoma susceptibility gene (*CDKN2A*) on chromosome 9.
- first to identify mutations of the *CDKN2A* gene in Australian melanoma families.
- first to identify mutations of the *MEN1* gene in Australian the multiple endocrine neoplasia type 1 families, and in sporadic endocrine tumours.
- first to publish linkage analysis of Australian breast cancer families.
- first to show that tumours from patients with the Beckwith-Wiedemann syndrome showed loss of somatic heterozygosity for markers on the short arm of chromosome 11, thus implicating this region as the location of a suppressor gene specific for these tumour types.
- first to show that pancreatic tumours from patients with MEN 1 showed loss of somatic heterozygosity for markers on the long arm of chromosome 11 around the *MEN1* gene.

- first in Queensland to develop human DNA fingerprinting.

FINANCIAL SUPPORT FOR RESEARCH:

CURRENT:

UQ-CRF 2000 $20000
Linkage disequilibrium mapping of a gene for mole development on chromosome 9p.
N. HAYWARD, N. MARTIN

QCF 2000 $52840
Epidemiological determinants of abnormal gene expression in cutaneous melanoma.
D. WHITEMAN, A. GREEN, N. HAYWARD

QCF 1999 $49512
2000 $50060
Development of mouse models of multiple endocrine neoplasia type 1.
N. HAYWARD, G. KAY, M. CUMMINGS.

NHMRC 1998 $133863 (NB: budget subsumed into institute block grant)
1999 $133863
2000 $133863
2001 $133863
Localization and cloning of genes for melanoma.
N. HAYWARD.

NHMRC 1997 $111170
1998 $112780 (NB: budget subsumed into institute block grant)
1999 $114388
Cloning and characterising the multiple endocrine neoplasia type 1 gene.
N. HAYWARD.

NHMRC 1996 $89388
1997 $93860
1998 $93860 (NB: budget subsumed into institute block grant)
Inheritance of susceptibility to melanoma.
J. AITKEN, N. MARTIN, N. HAYWARD, A. GREEN.

AMRAD 1/7/99 – 30/6/00 $137263
Characterization of *Vegfb* function *in vivo*
D. BELLOMO, A. MOULD, M. CAHILL, M. GARTSIDE, G. KAY, N. HAYWARD.

PAST:

QCF 1998 $56514
1999 $56514
In vivo analysis of Vegf-B function.
G. KAY, M. CUMMINGS, N. HAYWARD.

AMRAD 1/7/98 – 30/6/99 $133129
Characterization of *Vegfb* function *in vivo*
D. BELLOMO, M. GARTSIDE, G. KAY, N HAYWARD

QCF 1996 $52331
1997 $53950
1998 $53950
Analysis of p16 in control of cell cycle and melanoma tumorigenesis.
B. GABRIELLI, M. CASTELLANO, N HAYWARD.

AMRAD 1998 $25000
Molecular and functional characterisation of VRF/VEGF-B.
N HAYWARD, G. SILINS, S. GRIMMOND.

NHMRC 1994 $106169
1995 $107867
1996 $112457
1997 $116796
Localization and cloning of genes for familial melanoma.
N HAYWARD.

ARC 1997 $24000
Use of transgenic and knockout mice to study the physiological role of a novel growth factor.
G. KAY, N HAYWARD

AMRAD 1997 $50000
Molecular and functional characterisation of VRF/VEGF-B.
N HAYWARD, G. SILINS, S. GRIMMOND.

QCF 1996 $78921
Cloning the gene for multiple endocrine neoplasia type 1.
S. GRIMMOND, N HAYWARD.

NHMRC 1994 $106169
1995 $107867
1996 $109593
Localization and cloning of genes for familial melanoma.
N HAYWARD.

NHMRC 1994 $140081
1995 $124916
1996 $124916
A molecular genetic linkage study of schizophrenia.
B. MOWRY, N HAYWARD.

QCF 1994 $50040
1995 $51040
1996 $51040
Development of a superoxide dismutase 2 transgenic model for melanoma.
K. ELLEM, G. KAY, P. PARSONS, N HAYWARD

QLD UNI 1995 $20000
Role of the p16 gene in the development of melanoma.
N HAYWARD.

AMRAD 1995 $49949
Characterisation of genomic structure and biological activity of a novel growth factor.
N HAYWARD, S. GRIMMOND.

NHMRC 1993 $79061
1994 $79931
1995 $81210
Cloning of the multiple endocrine neoplasia type 1 susceptibility gene.
N HAYWARD.

QCF 1994 $57505
1995 $58660
Genetic predictive testing of breast cancer susceptibility.
N HAYWARD, N. WETZIG, I. BENNETT, B. TEH.

PAH 1993 $15000
1994 $15000
Genetic studies of breast cancer.
N. WETZIG, I. BENNETT, B. TEH, N HAYWARD.

QCF 1991 $37455
1992 $41842
1993 $42680
Linkage analysis of chromosome 6 markers in familial melanoma.
N HAYWARD.

QCF 1991 $46000
1992 $46000
Characterization of a centrosomal protein with an hTGFá related epitope.
K. ELLEM, N HAYWARD

NHMRC 1990 $103930
1991 $65316
1992 $72609
Chromosomal mapping of genes involved in familial and sporadic melanoma.
N HAYWARD, K. ELLEM.

QCF 1990 $37097
1991 $40065
1992 $42469
Characterization and molecular cloning of transcription factors for transforming growth factor genes.
N HAYWARD, K. ELLEM.

NHMRC 1990 $99433
1991 $104052
1992 $108098
Post UV excision of TGF∀: autocrine epidermal cell replication/tumour promotion.
K. ELLEM, N HAYWARD.

QLD UNI 1989 $25000
Oncogenes in hepatitis B virus associated hepatocellular carcinoma.
W. COOKSLEY, J. SCOTT, N HAYWARD.

QCF 1988 $50427
1989 $52948
Human melanoma: molecular genetics of tumour growth factors.
K. ELLEM, N HAYWARD, G. McLEOD, P. PARSONS, J. LITTLE.

QCF 1986 $48498
1987 $48498
Human melanoma: molecular genetics of tumour growth factors.
K. ELLEM, N HAYWARD, S. WILSON, C. KIDSON. G. McLEOD, P. PARSONS.

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COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure NKH-2** referred to in my Statutory Declaration made this 8th day of December 2000.

N. Hayward

Nicholas Kim Hayward

WITNESS: _____

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

DOCUMENT LIST

Documents provided to me by the Patent Attorneys representing HGS in the subject proceedings are as follows:

1. Cockerill, *et al.*, 1994, In: International Reviews of Cytology. A Survey of Cell Biology 159: 113-160
2. Maglione *et al.*, 1991, Proceedings of the National Academy of Sciences (USA) 88: 9267-9271.
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COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, **Jennifer Ruth Gamble** of the Hanson Centre for Cancer Research, in the Institute of Medical and Veterinary Science, Frome Rd, Adelaide, South Australia, 5000 declare as follows:

1. Scientific And Professional History.

1.1 I am the Co-Head of the vascular biology unit at the Hanson Centre for Cancer Research. I am also an Associate Professor with the Department of Medicine at the University of Adelaide, a position that I have held since 1998. I am a Research Hospital Scientist with the Institute of Medical and Veterinary Science in Adelaide and a foundation member and co-head of the vascular biology unit of the Hanson Centre for Cancer Research, which was established in 1989.

1.2 I received my Bachelor of Applied Science degree from the Royal Melbourne Institute of Technology, Victoria in 1978. I went on to receive my Masters of Science degree from The Walter and Eliza Hall Institute of Medical Research at the University of Melbourne in 1986. Subsequently

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I received my Doctorate of Philosophy from University of Adelaide in 1994.

1.3 My research studies over the last fifteen years have been in the general field of immunology and vascular biology. In particular, I have been studying the cells that form the lining of blood vessels and lymphatic vessels, known as the endothelium. These endothelial cells are involved in the processes of inflammation and new blood vessel growth, the latter process being termed angiogenesis. A specific area of interest has been the response of vascular endothelial cells to a group of molecules, termed growth factors, which affect the growth and behaviour of cells. In my research I have worked with many different growth factors including colony stimulating factors, interleukins and vascular endothelial growth factors ("VEGFs").

1.4 The study of angiogenesis is of enormous clinical significance because the growth of new blood vessels play an important role in biological processes such as wound healing and tumour development.

1.5 Nearly all cells in a vertebrate such as a mammal are located within 50μm of a capillary so as to receive oxygen and other nutrients from the circulatory system. Cells receiving insufficient oxygen usually release signalling proteins (eg growth factors) that stimulate the growth of blood vessels towards those cells. Thus, after wounding, a burst of capillary growth is stimulated in the neighbourhood of the damaged tissue to provide a new network of blood vessels to supply the healing tissue and to replace those blood vessels damaged by the injury. It is thought that the lack of oxygen to damaged tissue causes the release of growth factors by those cells, which triggers the angiogenic process. However, the process of angiogenesis takes time and can be insufficient or ineffective in certain situations. For example, ischemic damage takes place in catastrophic events such as heart attacks, strokes and angiogenesis is insufficient in other ischemic conditions such as in critical limb ischemia. Further, after surgery, the body's response may not be sufficient to promote satisfactory healing. Consequently, there is great interest in the development of a means to stimulate the body's natural angiogenic processes.

1.6 Tumours, like any other body tissue, also need a suitable blood supply. However, the growth of a solid tumour is often limited by its blood supply.

Consequently, a critical step in tumour progression is induction of capillary growth into the tumour mass. To do this, the tumour cells have to provide signals to existing capillaries to branch out and grow new vessels into the tumour. Unless a tumour is able to do this, it will be unable to grow more than a few millimetres in diameter. Tumours elicit new vessel growth by the nature of their ischemic environment and by secreting the same angiogenic growth factors as normal cells. There is, therefore, considerable clinical interest in elucidating the normal angiogenic process so that methods can be devised for inhibiting tumour growth by blocking the process.

1.7 Since about 1991, the vast majority of the work that I have done has involved cell biology and biochemical studies of endothelial cell function focussing on the regulatory effects of growth factors. I have authored or co-authored over eighty-five scientific papers in refereed publications. My research team and I have also been searching for novel factors involved in the process of angiogenesis. Since 1993 we have isolated a number of possible candidate molecules. The isolation of angiogenically important molecules has now developed into a major area of interest for my laboratory.

1.8 Examples of my research are described in many of my publications listed in my *curriculum vitae*. Now produced and shown to me marked "**Annexure JRG-1**" is a true copy of my *curriculum vitae*, which identifies the publications of which I have been an author or co-author.

1.9 Thus, as a Research Scientist, and subsequently co-head of the vascular biology unit, at the Hanson Centre for Cancer Research, I have conducted and directed an extensive amount of research in the field of angiogenesis and endothelial cell biology.

1.10 Now produced and shown to me marked "**Annexure JRG-2**" is a summary of the research that I have conducted over the last fifteen years.

1.11 Thus, I have extensive knowledge of the field of angiogenesis and endothelial cell biology, and its development in Australia over the last fifteen years. As such, I am familiar with the background knowledge, experience and technical abilities of researchers in my field, especially in Australia over the last fifteen years through my research, writing, supervisory responsibilities and referee duties for various international

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journals. I have been required to develop and maintain an extensive knowledge of the Australian and international scientific literature for a diverse range of scientific fields including molecular biology, biochemistry, cell biology, and angiogenesis etc.

1.12 In the following sections I refer to various scientific publications and patent specifications. Unless otherwise identified, I have not enclosed copies of these documents with this statutory declaration since the patent attorneys representing Human Genome Sciences Inc ("HGS") have informed me that copies of these documents will be filed by separate means.

2 My instructions

2.1 I am informed by the patent attorneys representing HGS that this proceeding concerns an opposition by Ludwig Institute for Cancer Research to Australian Patent Application Au-B-696764 (73941/94) in the name of HGS and entitled "Vascular Endothelial Growth Factor 2" ("the patent specification"), which has an earliest date of filing of 8 March 1994 ("March 1994"). I have been asked to provide my comments and opinions on the patent specification for use in these proceedings. My opinions concerning the content (information) in the patent specification are contained in this statutory declaration.

2.2 The patent attorneys for HGS have provided me with a copy of a document entitled "*Guidelines for Expert Witnesses in Proceedings in the Federal Court of Australia.*"

2.3 The patent attorneys for HGS have provided me with copies of various documents. Now produced and shown to me marked "**Annexure JRG-3**" is a list of those documents. I have been asked to review those documents and to provide my comments thereon. My decision not to address each passage in each of the statutory declarations that I have reviewed in a consecutive order should not be viewed or taken as an admission on my part of acceptance of any text that I do not comment on. Rather, I have found much of the evidence to be repetitive and have structured my comments in a manner to avoid excessive repetition.

3 Summary of The Patent Specification

3.1 The invention disclosed in the patent specification relates generally to vascular endothelial growth factor 2 ("VEGF-2"). Today, VEGF-2 is identified by the nomenclature VEGF-C. When I read VEGF-2 in the patent specification I understand it to mean VEGF-C. For the sake of consistency with the patent specification, however, I shall use the term VEGF-2 in this statutory declaration.

3.2 I would classify the general field of the invention described in the patent specification as the identification of a novel growth factor that is active in the process of endothelial cell growth and differentiation. In this statutory declaration I refer to that general field as the "field of endothelial cell growth and differentiation". Endothelial growth factors are particularly active in the process known as angiogenesis, i.e. the process leading to the formation of new blood vessels from pre-existing vessels.

3.3 I note that the specification is principally directed to a disclosure of a human VEGF-2 gene sequence and polypeptide sequences and its uses. The disclosure in the patent specification does not, however, end with that information since the specification also discloses recombinant techniques and procedures for producing polypeptides from VEGF-2 gene sequences. More specific detail concerning the information in the patent specification is provided in section 6 below.

4 The Field of Endothelial Cell Growth and Differentiation, including Angiogenesis in March 1994

4.1 I have been asked by the patent attorneys for HGS to comment on the state of knowledge of researchers working in the field of endothelial cell growth and differentiation, which includes angiogenesis, leading up to the development of the invention described in the patent specification, as I understood it to be at March 1994. In providing these comments I rely not only upon my research and experience, but also substantially rely on discussions I had prior to March 1994 with others in my field and the results of research and developments published by others in the field of vascular biology generally around the world in scientific journals. I assess published work by others for myself and if I consider it to be sound and relevant. use it as a basis of knowledge and assistance in my own research.

4.2 In providing my comments I have to the best of my ability endeavoured to be mindful not to take into account, unless otherwise stated, any information that I have subsequently obtained after March 1994.

Standard of Knowledge in Australia

4.3 In my opinion the standard of knowledge in the field of endothelial cell growth and differentiation, including angiogenesis, in Australia was equivalent to that anywhere else in the world as of March 1994. A number of Australian groups were actively working in the field in and prior to March 1994. By way of example, I consider that my work on the regulation of endothelial adhesion, in developing an improved gel-type assay capable of assaying for the biological activity of endothelial growth factors and my work on integrins, are some examples of work that was significant for this field.

4.4 Technology and scientific developments in the field of endothelial cell growth and differentiation both outside and within Australia are now, and were prior to March 1994, transmitted rapidly throughout the world through medical and scientific journals and publications, through telephone conversations with colleagues overseas, through computer links with overseas databases, from conferences held in Australia at which overseas researchers and practitioners made presentations, and from Australian researchers and practitioners returning from overseas conferences.

4.5 One of the ways in which I and others identified scientific publications in a field of interest was by the use of the *Medline* electronic database maintained by the National Library of Medicine in the United States of America and containing an index to the world's biomedical literature. Access to *Medline* in an on-line format has been available in Australia since before March 1994. The *Medline* database enables searches to be conducted of the medical and scientific literature for papers relating to particular topics of interest. I, and others with whom I have worked, have regularly used the *Medline* database as a research tool, as a matter of routine, to locate articles on particular topics since before March 1994.

4.6 In addition, many Australian researchers had done post-doctoral studies in overseas laboratories in institutions, as I had, and had kept connections with those laboratories and institutions so that a lot of

knowledge is and was transmitted by personal communication, often before publication. Some overseas researchers and practitioners also came to work in Australian laboratories and institutions.

4.7 Thus to summarise, in my opinion, the general state of knowledge in the area of endothelial cell growth and differentiation, including angiogenesis, in Australia in March 1994 would not have been greatly different from that in other developed and industrialised countries such as the United States of America and many countries in Europe. Based on my experience, the general level of skill of persons working in laboratories and research institutions in Australia would have been similar to that of persons working in overseas laboratories and institutions.

Background Knowledge in the field in March 1994

4.8 By March 1994 a lot of information was known about endothelial cell growth and differentiation, including angiogenesis. By way of example of the information that was known by my colleagues and me, I refer to two review articles that my colleagues and I wrote, in 1994 and in 1995 that described the general state of this field. Those articles were:

(a) Cockerill, Gamble, Vadas (1994) "Angiogenesis: models and model vectors". In: International Reviews of Cytology. A Survey of Cell Biology 159: 113-160; and

(b) Litwin, Gamble, Vadas (1995) "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129.

4.9 These review articles are identified as publications 58 and 59 respectively in my *Curriculum Vitae*. Although document (b) was published in 1995 the majority of the research reported concerns data published prior to 1994 and or that we were aware of prior to 1994.

4.10 Endothelial cells form the inner lining of the blood and lymphatic vessels of vascular systems. Endothelial cells play a critical role in physiological and pathological processes, including inflammatory responses, wound healing and the generation of new vessels.

4.11 The generation of vessels of the vascular system is termed vasculogenesis or angiogenesis. Vasculogenesis is mainly used to describe the *de novo* generation of vessels occurring during embryogenesis.

4.12 The term angiogenesis is routinely used to summarise a myriad of different cellular events that occur after vasculogenesis that lead to the development of new blood vessels through sprouting from pre-existing vessels. This process involves the migration and proliferation of endothelial cells from pre-existing vessels. Angiogenesis is not limited to the embryonic period of development but also occurs in adults where the formation of vessels is required and is of particular significance in wound healing, maturation of ovarian follicles and tumour development.

4.13 Angiogenesis is known to be controlled by the release of growth factors from neighbouring tissues. By 1994, many different growth factors, had been shown to exert effects on the growth, differentiation and behaviour of cells of the vasculature including endothelial cells, smooth muscle cell and fibroblasts and were, therefore, expected to participate in angiogenic processes in one way or another. Examples of such growth factors included vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and placenta-derived growth factor (PlGF).

Endothelial Growth Factors and related proteins – PDGF & VEGF

5.1 As discussed above, one of my primary research interests prior to and after 1994 was the role of growth factors in regulating endothelial cell function. Indeed, in 1995, my colleagues and I co-wrote a review article that summarised the role of various growth factors in endothelial cell function (Litwin, Gamble, Vadas, 1995, "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129). I refer to that review as a sample of the knowledge and information that was known by 1994 about these growth factors, including VEGF and PDGF.

(a) **PDGF**

5.2 Platelet-derived growth factor (PDGF) was identified in 1974 in human platelets as a potent growth factor for smooth muscle cells, fibroblasts and glial cells. Subsequent research established it to be a dimer. Cloning and sequencing of the two chains revealed that they are similar to each other and have been called PDGFα and β.

5.3 Both PDGFα and β possess N-terminal hydrophobic signal sequences that can direct secretion of the protein from the cells. Further, both forms are proteolytically processed to form the mature protein. PDGFα and β both contain the eight cysteine residues and 14 amino acid signature motif characteristic of all members of the PDGF/VEGF family.

5.4 Prior to 1994, PDGF was shown to stimulate the growth of connective tissue cells including smooth muscle cells. In addition to inducing cell replication, PDGF was also shown to elicit a number of intracellular signals related to mitogenesis, to be chemotactic, to act as a vasoconstrictor, to activate leukocytes, and to modulate extracellular matrix turnover. PDGF had also been shown to be involved in the regulation of cell growth and chemotaxis during embryonic development.

5.5 Two different types of receptor for PDGF have been identified, the alpha and beta receptors. They are both receptor tyrosine kinases.

(b) **VEGF**

5.6 Vascular endothelial cell growth factor (VEGF), was isolated in 1987 from foetal bovine retina and was shown to be highly specific for endothelial cells, by contrast to other known growth factors such as PDGF which affect a range of cell types.

5.7 In 1989 cDNA clones for both human and bovine VEGF were obtained. Analysis of the primary amino acid sequence of VEGF revealed some homology to the α and β chains of PDGF (18 to 20%). In particular, VEGF was shown to contain the same eight conserved cysteine residues that are also present in PDGF. VEGF also contains the 14 amino acid signature motif characteristic of all PDGF/VEGF family members.

5.8 VEGF was known to comprise a hydrophobic N-terminal signal sequence that directs secretion of the protein. VEGF, like PDGF, is proteolytically

processed to form the mature protein. Further, all forms of VEGF were known to exist as dimers, joined by disulphide bonds. However, unlike PDGF, the polypeptide making up the dimers of VEGF are identical.

5.9 The secreted forms of VEGF were demonstrated to promote the growth of vascular endothelial cells but not others, which contrasted to that seen for PDGF.

5.10 VEGF has been shown to increase blood vessel permeability, stimulate proliferation in vascular endothelial cells and promote angiogenesis. VEGF has also been shown to be expressed at high levels in a number of tumours and to confer Chinese Hamster Ovary (CHO) cells with the ability to form tumours in nude mice. These observations provided evidence for a role for VEGF in promoting the growth of new blood vessels during tumour development: the ability of solid tumours to stimulate the growth of new blood vessels is an essential part of their development.

5.11 In 1991, a receptor for VEGF was characterized, the Flt-1 receptor (also known as VEGFR-1). A further VEGF receptor, the KDR/Flk-1 receptor (also known as VEGFR-2) was identified in 1993. Both receptors are members of the receptor tyrosine kinase superfamily. Binding of VEGF to the receptor results in receptor phosphorylation.

(c) PlGF

5.12 Placenta growth factor (PlGF) was identified in 1991 from a placenta cDNA library. It is a 149 amino acid protein comprising a N-terminal hydrophobic signal sequence and proteolytic cleavage sites. Computer analysis revealed 53% identity to VEGF over the conserved PDGF-like region that comprises eight cysteine residues and the 14 amino acid signature motif characteristic of all members of the PDGF/VEGF family. Expression of the cDNA encoding PlGF resulted in a secreted protein in the form of a dimer of two identical chains. The recombinant protein was also shown to be capable of stimulating the growth of endothelial cells but not NIH3T3 fibroblast cells. This finding indicated that recombinantly produced PlGF protein had the same specificity for endothelial cells as VEGF.

5.13 Thus, PDGFα, PDGFβ, VEGF and PIGF are all secreted growth factors. Each of these proteins contains an N-terminal hydrophobic signal sequence and is proteolytically processed into a mature form. They all form dimers, either with themselves or with related proteins. Each of these proteins contains eight cysteine residues and the 14 amino acid signature motif characteristic of the PDGF/VEGF growth family, and they are all involved in angiogenesis.

Angiogenic Assays

5.14 The preceding paragraphs provide a summary of the known (i.e. by 1994) properties and biological activities of the PDGF/VEGF family of growth factors involved in angiogenesis. These properties and activities were determined using a number of bioassays, routinely used prior to 1994. These assays allowed my research team and others not only to distinguish between the various growth factors but also to explore further the biological properties of any growth factor of interest. For example, many of the bioassays allow direct determination of mitogenic and angiogenic activities and permit repeated, long-term quantitation of mitogenesis and angiogenesis as well as physiological characterization of angiogenic vessels. Some of the assays that were routinely available and used by my research team and others in the field, before 1994, are discussed in more detail below.

5.15 In 1994, common *in vitro* and *in vivo* assays available for measuring endothelial cell associated activity included:

(a) *In vitro* anglogenic assays

i. Proliferation assay
ii. Three Dimensional Gel assay
    1. Type I Collagen gels
    2. Matrigel
    3. Fibrin gels
iii. Wound type assay
iv. Permeability assay
v. Aortic Ring model

(b) *In vivo* angiog nic assays

i. Chicken Chorioallantoic Membrane assay

ii. Corneal Neovascularisation assay
iii. Pouch assay

5.16 Rather than provide an expansive discussion on each of the available assays in this Statutory Declaration I refer to Litwin, Gamble, Vadas, 1995, "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129, which my colleagues and I wrote in 1994, which provides details of a number of the assays listed above.

5.17 Prior to 1994 my laboratory staff and I routinely used three different assays to measure aspects of angiogenesis *in vitro*. They were the cell proliferation assay, three dimensional gel assay (which I improved in my laboratory, see below) and a wound assay. Many research teams around the world also routinely used these assays. Using these assays my team and I examined the angiogenic activity of a number of growth factors including endothelial growth factors such as VEGF.

5.18 Between 1992 and 1993 I also developed a high throughput *in vitro* angiogenic assay capable of assessing large numbers of angiogenic molecules. That assay was reported in Gamble J.R. *et al.*, 1993, Journal of Cell Biology 121: 931-934. Journal of Cell Biology is one of the standard journals in my field of research and one that my colleagues and I would routinely read.

5.19 The high throughput assay that we developed significantly improved existing three dimensional gel assays because it provided a means to test many potential angiogenic molecules at once in a routine and standard manner. This assay was routinely being performed in my laboratory prior to March 1994 and in fact provided the means by which my research team were able to assay or screen large numbers of monoclonal antibodies for anti-angiogenic activity.

5.20 In addition to the tests that my staff and I were conducting as part of our research prior to 1994, we also provided some support to various researchers in South Australia and some researchers in other states of Australia, by testing for the presence of angiogenic activity in samples of material that they provided to us.

5.21 Although my research team and I never established any *in vivo* assays to measure aspects of angiogenesis, I was aware of a number of research groups around the world that were using such assays. At that time our research did not require the use of these assays since we were concerned with the action of factors directly on the endothelial cells rather than the overall process of angiogenesis that takes place *in vivo*. However should our work have evolved in such a direction then we would have established the relevant models. Many of these *in vivo* assays, although known to be labour intensive, once established could be performed by a competent research assistant.

5.22 In summary, prior to 1994, there were a number of assays available that could be, and were, routinely used to test the activity of putative and known angiogenic growth factors such as VEGF and PDGF.

6. The patent specification

6.1 I note that HGS succeeded in identifying a VEGF-2 gene and polypeptide sequence. In particular, the patent specification discloses that HGS identified a 1525 base pair nucleotide sequence, which they identified as translating to a 350 amino acid sequence (see: Figure 1 in the patent specification).

6.2 The patent specification teaches that the disclosed sequence contains the fourteen amino acid signature motif that is common to the PDGF/VEGF family of growth factors, as well as the eight cysteine residues which are conserved amongst VEGF, PDGFα, PDGFβ and PlGF family members (see paragraphs 5.1 to 5.13, above). By 1994, I knew from my own studies conducted on VEGF and PDGF, in addition to work published by others, that the signature motif and the cysteine residues in VEGF and PDGF were important for defining the biological activity of these molecules. Further, I was aware that the cysteine residues enable the PDGF/VEGF family of proteins to form dimers, which contributes to the biological activity of the molecule.

6.3 By March 1994 each of VEGF and PDGF were both known to undergo some proteolytic cleavage in their passage out of the cells in which they are produced. In particular, both molecules were known to be produced with a signal sequence which aides the passage of the molecules out of the cell. The processing of these molecules was also known to occur as

they are released from the cell and is important for producing a biologically active molecule.

6.4 I note that a portion of the full length VEGF-2 sequence, which is not disclosed in the patent specification, is part of the NH_2 (amino) terminal end of the full-length polypeptide sequence. This equates to 69 amino acids at the amino terminal end of the VEGF-2 polypeptide sequence. In all secreted proteins the signal or leader sequence is located at the amino terminal end of the molecule. VEGF-2 is no different in that it contains a signal or leader sequence at its amino end. This sequence is located among the 69 amino acids that were not disclosed in the patent specification.

6.5 The mere fact that the sequence disclosed in the patent application does not include the first 69 amino acids of the full-length VEGF-2 sequence would not dissuade me from attempting to express the sequence disclosed in the patent specification with a heterologous signal sequence. I would expect that the expressed protein would be secreted and biologically active. Although by 1994, the proteolytic processing of VEGF-2 had not been elucidated, the processing of related proteins VEGF and PDGF was well characterized. By 1994, it was known that members of the PDGF/VEGF family were proteolytically processed as they were secreted from the cell. Thus, I would predict that VEGF-2 would be expressed in a similar way. Additionally, the, biologically active forms of PDGF/VEGF proteins were known to retain their characteristic eight cysteine residues and 14 amino acid signature motifs. Thus, as the sequence disclosed in the patent specification contains both of these domains important for biological activity, I would expect that it would be correctly processed by the cell and secreted as a biologically active VEGF-2 protein. Dr Alitalo's own work has clearly confirmed that the sequence set forth in the patent specification combined with a heterologous signal sequence confers sufficient information to encode a secreted biologically active protein. (See: U.S. Patent No. 6,130,071, issued October 10, 2000, to Alitalo et al., column 47 line 57 to column 48 line 2).

Significance of the contribution provided by the patent specification

6.6 In my opinion, the identification of the DNA sequence encoding VEGF-2 and the inherent information it provides, combined with the teachings of

the patent specification, makes possible the manifest therapeutic benefits, which VEGF-2 has to offer, and which will be obtained in the future. The invention described in the patent specification embraces products and processes which have the potential to provide, and will continue to provide, therapeutic benefits to the public. The patent specification also provides the information necessary for production of a recombinant product with one or more of the biological properties of VEGF-2. It discloses methods for constructing various expression vectors suitable for use in a range of prokaryotic and eukaryotic host cells (*see e.g.*, pages 10 to 15 of the patent specification). It discloses host cells for expression, including eukaryotic mammalian cells, eukaryotic yeast cells, insect cell, and prokaryotic cells (*see e.g.*, pages 10 to 15 of the patent specification). By March 1994 all of this information was routinely available from standard text.

6.7 Further, I note that the patent specification teaches *in vitro* assays which I could have used as an assay system for testing for the biological function or activity of any protein I produced from the description in the patent specification. For example, on page 18 lines 6 to 8 the specification suggests assaying VEGF-2 by its ability to proliferate endothelial cells. This type of assay was so routine and well known in the field by 1994 that it was not necessary to provide any further details. Thus, the patent specification coupled with the state in the art as of March 1994 would enable one of skill in the art to assay to biological function or activity of VEGF-2. The patent specification did not need to describe specific experimental protocols to enable me to test for angiogenic activity in a recombinant protein.

6.8 In summary having regard to the state of knowledge associated with PDGF/VEGF proteins in March 1994, I believe the patent specification provides, amongst other information, the following:

6.8.1 The amino acid sequences of a range of different length VEGF-2 polypeptides;

6.8.2 The precise DNA sequence encoding the amino acid sequences identified in sub-paragraph 6.9.1;

6.8.3 Information identifying where VEGF-2 could be isolated from (eg a human cell library derived from human embryo (week 8 to 9) osteoclastomas, adult heart or breast cancer cell lines);

6.8.4 Methods for producing recombinant VEGF-2 in eukaryotic and prokaryotic host cells;

6.8.5 Ways for producing abundant amounts of a polypeptide isolated from recombinant cells having the *in vivo* activity of VEGF-2;

6.8.6 VEGF-2 polypeptide analogues;

6.8.7 VEGF-2 polypeptide antagonists, VEGF-2 polypeptide agonists;

6.8.8 VEGF-2 polypeptide antibodies;

6.8.9 Ways to test for the *in vitro* or *in vivo* biological function or activity of VEGF-2;

6.8.10 Pharmaceutical carriers and delivery systems for the VEGF-2 polypeptide as well as information concerning gene therapy to provide therapeutic and prophylactic effects against a wide range of different disease states; and

6.8.11 Uses of the VEGF-2 polypeptide and gene sequence for the treatment of numerous medical conditions.

6.9 At the conclusion of the patent application there are 61 claims, which define various aspects of the information that is described in the patent application.

Signal sequence

6.10 In March 1994 I was aware that angiogenic growth factors were secreted (such as the PDGF/VEGF family of growth factors including PDGFα, PDGFβ, VEGF and PlGF). Further, I knew that before an angiogenic protein could exert an angiogenic influence on a cell it needed to be released from the cell cytoplasm in which it was produced. Release of members of the PDGF/VEGF family was brought about by secretion of the respective member proteins.

6.11 Given that VEGF-2 was structurally similar in many ways to VEGF, PDGFα, PDGFβ and PlGF (eg it has eight cysteine residues and a 14 amino acid signature motif which is characteristic of these molecules (see: the patent specification page 5)), I would have expected that VEGF-2 was also a secreted protein.

Ludwig's Evidence in Support

7.1 I have been asked to read and comment on some of the statutory declarations by Ludwig's witnesses.

7.2 I have read:

7.2.1 The Statutory Declaration of Peter Adrian Walton Rogers executed on 16 February 2000 (Associate Professor Rogers' Statutory Declaration);

7.2.2 The Statutory Declaration of Kari Alitalo executed on 15 February 2000 ("Dr. Alitalo's Statutory Declaration"); and

7.2.3 The Statutory Declaration of Francis John Ballard executed on 16 February 2000 ("Dr. Ballard's Statutory Declaration").

7.3 In the following paragraphs I comment on some general issues raised in the above Statutory Declarations. I then turn to some specific comments made by each declarant. My decision not to address each paragraph in each Statutory Declaration should not be taken as an admission of acceptance of the paragraphs to which I do not refer.

7.4 Collectively, the declarants appear to suggest that the claims in the patent application cover molecules like VEGF, PDGFα, PDGFβ and PlGF. They appear to me to suggest that expression of the VEGF-2 sequence would have been problematic because of a variety of theoretical problems, none of which however appear to me to bear any relation to the case at hand.

7.5 There are essentially three issues raised by the Applicant's experts affidavits:

7.5.1 That the claims in the patent application cover molecules like VEGF, PDGFα, PDGFβ and PlGF because of the use of the words "fragments, analogues and/or derivatives";

7.5.2 That the patent specification fails to disclose a biological assay for testing for VEGF-2 or show biological activity of the identified VEGF-2 polypeptide sequence; and

7.5.3 That the patent specification does not identify a single antibody and conserved sequences between VEGF-2 and other PDGF/VEGF family members may bind cross reactive antibodies.

7.6 I deal with each of these issues in turn.

The claims in the patent application do not cover molecules like VEGF, PDGFα, PDGFβ and PlGF.

7.7 Reference is made on page 9, line 14 to page 10, line 5 of the specification to the meaning of the terms "fragment", "derivative" and "analog". When I read these passages I understood HGS to be saying that a fragment, derivative or analogue is a polypeptide that retains essentially the same biological function or activity as VEGF-2. While I acknowledge this basic requirement I am conscious of the fact that before something can be an analogue, fragment or a derivative of a protein it needs to share sufficient identity with that protein to make it resemble that protein or at least part thereof. In my opinion both of these requirements must be satisfied before a sequence of amino acids can truly be called an analogue; fragment or a derivative of VEGF-2.

(a) The need for biological function or activity

7.8 Provided a fragment, analogue or derivative has a biological function or activity then it should have some biological function or activity that is essentially the same as that observed for VEGF-2. When I read biological function or activity I immediately understand it to include at least *in vivo* and/or *in vitro* activity.

7.9 Testing for *in vivo* activity or *in vitro* activity of an angiogenic molecule like VEGF-2 would have been a routine exercise prior to March 1994. It was something that my laboratory could have easily conducted by 1994, since we were testing other angiogenic molecules like VEGF for *in vitro* activity.

7.10 There were a number of different assays available by 1994 capable of testing for VEGF-2 biological function or activity. For assays described in the specification I refer to and repeat paragraphs 5.14 to 5.22, above. For other assays that could have been performed to test for VEGF-2 biological function or activity I refer to and repeat paragraph 4.8 above.

7.11 As an additional point of distinction between VEGF-2 and PDGFα, or PDGFβ is that PDGFα and PDGFβ do not promote the growth of endothelial cells. Thus had I wanted to distinguish a VEGF-2 fragment, analogue or derivative from any one of these proteins, I could have easily

performed one or more assays, such as those identified in paragraph 5.15, above. Such assays could be performed to show that the analogue, fragment or derivative contained an endothelial cell proliferative activity, while the comparator molecule (such as PDGFα and PDGFβ) did not contain such an activity. Such a result would clearly have distinguished a VEGF-2 analogue, fragment or derivative from PDGFα, or PDGFβ.

(b) Must look like VEGF-2 at the primary amino acid level.

7.12 For something to be a fragment, derivative or analogue of a molecule it must not only share a biological function or activity with VEGF-2 but it must also have homology at the primary amino acid level. Thus, a peptide or polypeptide that more closely resembles VEGF, PDGFα, PDGFβ or PlGF, than VEGF-2 at the primary amino acid level would not be a VEGF-2 fragment, derivative or analogue. Such an interpretation is inherent in the meaning of these terms and is consistent with the general manner in which others and I use these terms in everyday scientific language.

7.13 When regard is had to the information provided in the patent specification one observes that the homologies at the amino acid level between VEGF, PDGFα, PDGFβ and VEGF-2 are very low. The identity between VEGF-2 and each of VEGF, PDGFα, PDGFβ is 30%, 23% and 22% respectively (see page 5 of the patent specification). Further, there are very few contiguous sequences of amino acids that are identical between VEGF-2 and each of VEGF, PDGFα or PDGFβ (see Figure 2 of the patent specification) In fact, the largest single contiguous sequence of amino acids is only seven amino acids in length and is found in the signature motif, which is identified by a box in Figure 2. After that stretch of residues there is one other stretch of six contiguous amino acids but no other significant contiguous amino acid sequences that share identity between VEGF-2 and VEGF, PDGFα or PDGFβ. Thus, at the primary amino acid level there are significant differences between VEGF-2 and VEGF, PDGFα or PDGFβ.

7.14 I would not have any problem in distinguishing between a fragment, derivative or analogue of VEGF-2 and a fragment, derivative or analogue VEGF, PlGF, PDGFα or PDGFβ especially taking into account both biological activity and sequence homology. If the fragment, analogue or derivative has a higher homology to VEGF-2 than VEGF, PlGF, PDGFα or PDGFβ and shares a biological activity also present with the sequence disclosed in the patent application then I would consider it a VEGF-2 fragment, analogue or derivative.

The patent specification provides sufficient information to test for VEGF-2 biological function or activity.

7.15 The patent specification clearly identifies that VEGF-2 has angiogenic activities, and describes *in vitro* angiogenic assays of VEGF-2, which could be routinely used. Furthermore, by 1994 assays for angiogenic biological activity were widely reported in the literature. In this respect I refer to Litwin, Gamble, Vadas, 1995, "Role of growth factors in endothelial cell functions." In: Human Growth factors: Their role in Disease and Therapy, BB Aggarwal & RK Puri (Eds), Blackwell Science, Inc. USA Chapter 7 101-129, which identifies many publications that were available prior to 1994 that describe assays for testing many different angiogenic activities.

7.16 Had I been provided with a copy of the patent specification in March 1994 I would not have required anyone to tell me how to carry out an assay to test for an angiogenic activity. I refer to and repeat paragraphs 5.14 and 5.22 above which address this is in more detail.

7.17 I note that Ludwig's experts and in particular Associate Professor Rogers observe that HGS has not tested the biological activity of the protein that they describe in their patent application. However, confirmation that the VEGF-2 sequence described in the specification is expressed *in vivo*, and therefore likely to indicate a biological activity, is provided in the Examples of the specification where it is shown by Northern blotting that a number of malignant breast tumour cell lines over express VEGF-2. The over expression of VEGF-2 in breast cancer cell lines while absent in a normal breast sample suggests a role of VEGF-2 in tumor development perhaps by promoting the growth of new blood vessels, as was observed for VEGF. I refer to and repeat paragraphs 5.10. Other assays to further characterise biological activity, such as *in vitro* tests using purified VEGF-

2 protein, would have been straight forward for any laboratory that was set up to perform such assays. I refer to and repeat paragraphs 5.14 to 5.22 above which address this is in more detail.

7.18 Further, whether or not the patent specification gives examples of biological activity would seem to me to be largely irrelevant. If I were told that a molecule had angiogenic activity but there were no experiments present in that document to support that statement I would, in March 1994, have proceeded to test the molecule myself. Moreover, even if such data were provided I would probably still have conducted such experiments, because they were simple to carry out and routinely performed in my laboratory.

The patent specification provides sufficient information for VEGF-2 antibodies.

7.19 Associate Professor Rogers' asserts that the patent specification fails to indicate that the inventors actually made any anti-VEGF2 antibodies.

7.20 I believe the patent specification provides sufficient information for a person of ordinary skill to produce VEGF-2 antibodies (see pages 22 to 24). Methods for generating antibodies against a protein were very well known by March 1994. The patent specification identifies on page 23 a number of publications that describe such methods that were well known to me and had been used in my research prior to March 1994. In my opinion, a person of ordinary skill in the field of immunology should be able to produce antibodies against VEGF-2 with ease.

Cross reactivity of VEGF-2 antibodies with other proteins

7.21 Associate Professor Rogers appears to suggest (see, for example paragraph 2.7.16) that antibodies that bind to regions of VEGF-2 that are conserved with other PDGF/VEGF family members might be cross-reactive. Associate Professor Rogers refers to a number of prior art disclosures of anti-VEGF or anti-PDGF antibodies. Simply because two sequences share some homology does not necessarily imply that any antibody produced against one molecule would necessarily be cross-reactive against the other. In my opinion no conclusion can be made as to whether sequences sharing some homology will generate antibodies that are cross reactive.

(I) Associate Professor Rogers' Statutory Declaration

7.22 I refer to Associate Professor Rogers' Statutory Declaration, and make the following comments.

Paragraph 1.5.3

7.23 In paragraph 1.5.3, Associate Professor Rogers' states:

> "...extensive proteolytic processing occurs at both the amino- and carboxyl-terminal ends of the polypeptide, resulting in a much smaller circulating polypeptide that has enhanced and/or new biological activities relative to the larger pre-processed forms from which it was derived... ...However, in the opposed application filed by 1994, there is no evidence presented of complex proteolytic processing."

7.24 However, I was aware that all members of the PDGF/VEGF family (known in March 1994) underwent some proteolytic processing when produced from a cell. Thus, had I been asked to examine VEGF-2 in March 1994 I would have expected that VEGF-2 may also naturally undergo some proteolytic processing when released from a cell. This process occurs naturally during secretion, controlled by cellular enzymes. Consequently, if a researcher were able to produce VEGF-2 and secrete it from a cell I believe a reasonable expectation would be that processing would take place. Therefore, in my opinion, the patent specification fully enables one to express and secrete a biologically active protein that has been correctly processed.

Paragraph 2.2

7.25 In paragraph 2.2, Associate Professor Rogers' mentions that several claims in the patent specification are directed to a "fragment, analogue or derivative" of a VEGF-2 polypeptide (*e.g.*, claim 28) or to a polynucleotide encoding a "fragment, analogue or derivative" of a VEGF2 polypeptide (*e.g.*, claims 1 to 4 and 21). He then proceeds to state:

> "...all of the differences between the VEGF2 sequence and prior art sequences such as the human VEGF sequence can properly be characterized as modifications that involved substituting, adding, or deleting residues, the same types of modifications taught in the opposed application for making fragments, analogues, or derivatives."

7.26 I disagree with Associate Professor Rogers' statement. If a molecule has higher homology to VEGF than VEGF-2 then it is not in my opinion an analogue of VEGF-2.

7.27 Thus, I would not regard VEGF or PDGF or PlGF to be an analogue or derivative of VEGF-2, nor do I believe that a researcher of reasonable skill would have any problem in distinguishing between VEGF and VEGF-2 or a fragment, analogue or derivative thereof.

Paragraph 2.3.3

7.28 In paragraph 2.3.3, Associate Professor Rogers' states:

> "For example, there is no description of tests that were performed to demonstrate that VEGF2 has a biological activity that is useful in such procedures, and there is no description of any assay to test for VEGF2 biological activity."

7.29 As discussed in paragraph 7.17 above, I consider the Northern blots described in the Examples of the specification to be indicative of a biological activity for VEGF-2. Further, I do not agree that the patent application does not identify a means to test for VEGF2 biological function or activity. In this respect I refer to page 18 of the patent specification where I note the patent specification clearly states that VEGF-2 can be used for *in vitro* culturing of vascular endothelial cells. I recognise this as the basic cell proliferation assay and could have used this assay to test for VEGF-2 activity. It was not, however, the only assay available at that time for testing activity of molecules, like VEGF-2. There were many other assays some of which are described in paragraph 5.15 above.

Paragraph 2.7.18

7.30 In paragraph 2.7.18, Associate Professor Rogers' comments on the scope of claim 50. I particular he states:

> "Assuming *arguendo* that VEGF2 as taught in the specification possesses any biological activity that is mediated through cell surface receptors, the claims directed to antagonists of VEGF2 are not novel over prior art disclosures of forms of the receptors to which VEGF2 could bind, but could not signal. See **Document D27** (disclosing a dominant negative Flk-1 protein)."

7.31 When I read claim 50 I note that it requires the defined antagonist to be specific for the polypeptide of claims 28 to 48. Associate Professor Rogers then cites Document D27 that describes a dominant negative Flk-1 protein. There is no evidence in that publication, which establishes that the identified truncated receptor is capable of binding specifically to VEGF-2. I am aware from research published since 1994 that the Flk-1 receptor, when produced in a non-truncated form, binds VEGF as well as VEGF-2. Thus the truncated Flk-1 protein described in Document D27 is not specific for VEGF-2, as the claim appears to me to require.

Paragraph 2.7.19

7.32 In paragraph 2.7.19, Associate Professor Rogers' states:

> "Because of this alleged equivalence of activities taught in the opposed application, patients in need of VEGF2 (or in need of inhibiting VEGF2) would be treatable with VEGF (or with VEGF antagonists). Consequently, claims 51 and 52 embrace any prior art method of treatment of patients with VEGF polypeptides that are encompassed by claim 28 (or prior art method of treatment with VEGF antagonists)."

7.33 As I read claims 50 and 51 I note that they are directed to a method of treatment of a patient having need of VEGF2 or need to inhibit VEGF2 by administering an effective amount of a polypeptide according to claim 28 or an antagonist against a polypeptide of claim 28. I do not understand how either of these claims could possibly embrace the subject matter mentioned by Associate Professor Rogers. In particular, in my opinion the claims in the patent specification appear to be directed to use of VEGF-2 not VEGF and to the treatment of a VEGF-2 disorder. While VEGF-2 and VEGF may be used to treat some of the same disorders I note that HGS appear to me to have only claimed the use of VEGF-2.

Paragraph 2.7.20

7.34 In paragraph 2.7.20, Associate Professor Rogers' states:

> "I observe that the opposed application fails to identify with particularity any fragment, analogue, or derivative of the Figure 1 polypeptide which has an inhibitory activity."

7.35 I and I believe other researchers of ordinary skill in the molecular biological field could easily generate fragments, analogues or derivates of VEGF2 and could have tested them at March 1994 using any one of a

large number of different biological assays to detect inhibitory activity. In fact, in collaboration with other groups in the Hanson Centre for Cancer Research, we had been doing similar experiments at that time although not with VEGF2. Therefore, by providing VEGF-2 coding sequences, the patent specification provided all of the information necessary for me and I believe any one else of ordinary skill to identify a fragment, analogue, or derivative of the Figure 1 polypeptide that has an inhibitory activity.

Paragraph 4.6.5

7.36 In paragraph 4.6.5, Associate Professor Rogers' states:

> "To summarize, the opposed application contains no demonstration of VEGF2 activity and there is no reasonable basis upon which one skilled in the art could have predicted VEGF2 activity."

7.37 I refer to and repeat paragraphs 5.14 to 5.22, 7.8 to 7.11 and 7.15 to 7.18, which address this issue.

(ii) Dr. Alitalo's Statutory Declaration

7.38 I refer to Dr. Alitalo's Statutory Declaration, and make the following comments.

7.39 In 1996, Dr Alitalo first described a VEGF-C polypeptide sequence that was 350 amino acids long, not unlike the sequence provided in the patent specification. A copy of Dr Alitalo's publication is identified as **Document D70,** in his statutory declaration. In that publication the authors state on page 291 (first column):

> "The two longest clones of 2.0 and 1.8 kb contained an open reading frame (ORF) of 350 residues shown in Figure 3B, having two possible methionine codons (marked in bold) for translation initiation and a putative secretory signal sequence peptide (underlined) followed by the N-terminal sequence of the purified Flt4 ligand (marked in Bold)".

7.40 Further, on page 293 (first and second columns) the author's state:

> "Interestingly, the VEGF-C ORF is 350 amino acid residues long and our N-terminal sequence analysis confirmed that its putative signal sequence is removed before secretion."

7.41 Shortly after publishing these results Dr. Alitalo published a correction to that paper (see Joukov *et al.*, 1996, EMBO Journal 15: 1751). Thus, at the time of writing his statutory declaration, I would have expected Dr. Alitalo to be aware of the correct signal sequence from VEGF-C. Since he followed a similar path to that which HGS seems to have followed in the patent specification when attempting to characterise what he thought was the full length VEGF-2 sequence, he knew that the signal sequence identified in the specification was not such a sequence.

Paragraph 6.5

7.42 Dr. Alitalo's experimental protocol indicates that he employed two constructs, one encoding amino acids 1 to 419 of VEGF-C (VEGFC), and a second encoding amino acids 70 to 419, followed by an HA tag (VEGF2 (HGS)). In paragraph 6.5 of his declaration, Dr. Alitalo indicates that he used a monoclonal anti-HA antibody to detect VEGF2(HGS) and another antibody serum, 882 antiserum, which Dr Alitalo indicates is a polyclonal antiserum generated against residues 35-51 of the 350 amino acid VEGF2 polypeptide to detect VEGF-C.

7.43 I do not believe that any conclusions can be drawn from the experiments conducted by Dr. Alitalo because he used different antibodies to examine the VEGF2(HGS) protein and VEGF-C. The scientifically most meaningful method used to compare the secretion of VEGF2(HGS) protein and VEGF-C would be to use the identical antibody to immunoprecipitate the two proteins. Since the antibody used to immunoprecipitate VEGFC should also recognize VEGF2 then this should have been used. Alternatively, if the method of choice was to immunoprecipitate via a HA tag, then this tag should have been incorporated into both VEGF-C and VEGF2.

7.44 The second problem with the design of the experiments is a comparison made between HA tagged and non-HA tagged proteins. It has not been demonstrated that the HA tagging has not altered the capacity of the protein to be synthesised and secreted or detected. Indeed, in his own publication (Joukov et al., 1997, EMBO J. 16:3898-3911) Dr Alitalo and his colleagues have stated that they were unable to immunoprecipitate secreted VEGF-C with an antibody directed to a HIS-tag incorporated into the very C terminal end of the protein although they were able to do this when a HIS-tag was placed immediately behind the signal sequence.

Given the information now known and known at the time of designing this experiment it would appear to me that VEGF C/2 is highly processed with the very C terminal end of the protein removed.

7.45 In HGS' Australian Patent No. 714484, a monoclonal anti-HA antibody was used to successfully immunoprecipitate VEGF-2 which had been modified to contain an HA epitope at its carboxy terminus. It is unclear to me why Dr. Alitalo apparently was not able to isolate VEGF-2 using a His tag at the C-terminus. One explanation may be the type of mammalian cell line used in the experiments. The modified VEGF-2 was expressed from COS cells in HGS' experiments. However, Dr. Alitalo used 293T cells in the experiments reported in his declaration. The significance of the different cell types used by HGS and Dr. Alitalo can be found in Dr. Alitalo's own publication (Joukov et al., 1997, EMBO J. 16:3898-3911). This publication describes the proteolytic processing of VEGF-2 when expressed by a number of different cell lines, including COS cells, PC-3 cells, HT1080 cells and 293-EBNA cells. The result of this comparison was that "[t]he proteolytic processing of the VEGF-C precursor in COS cells was less efficient when compared with other cell types" (see: Joukov at page 3901, second column). Thus, as the VEGF-2 precursor is processed less efficiently in COS cells, one would also expect that the cleavage of the HA tag from the carboxy terminus would also be less efficient in COS cells, as compared to 293T cells. So, that the lack of efficient cleavage of the HA-tag from the protein may account for HGS' successful detection of the protein utilizing the HA-tag from COS cells, while Dr. Alitalo was unable to do so.

7.46 Assuming, however, that the ultimate conclusion reached in these experiments is correct, i.e. there is no secretion signal sequence in the 350 amino acid sequence disclosed in the patent specification, such a result would not have stopped me from attempting to produce a biologically active VEGF-2 polypeptide sequence using the information in the patent specification and knowledge available to those skilled in my field by March 1994.

7.47 Using the knowledge that PDGF/VEGF family members are secreted molecules and using the sequence information in the patent specification I would have reasoned that the failure of the VEGF-2 protein to be secreted may be due to an inefficient or atypical signal sequence or a

missing or partial signal sequence. Another possibility is that the VEGF-2 protein is indeed not secreted. However given that all the other PDGF/VEGF family members known prior to March 1994 were secreted molecules, I would have regarded this as an unlikely explanation.

7.48 Given the weight of evidence that VEGF-2 is indeed a secreted protein, I believe a more reasonable explanation for the lack of secretion of VEGF-2 is an inefficient signal sequence or a missing or partial signal sequence. Thus, this explanation would have prompted me to attach a strong signal sequence upstream from the nucleotide sequence encoding VEGF-2, as taught by the patent specification (at page 14 lines 6-23). Using this approach I would have fully expected to achieve expression and secretion of the VEGF-2 protein. Furthermore, as discussed above in section 6, the VEGF-2 sequence provided in the patent specification provides all the information required to encode a biologically active protein. Thus I would have proceeded using the approach provided by the patent specification and having done so, I would fully expect the VEGF-2 protein to be secreted and biologically active.

7.49 In addition, since as mentioned above, the lack of secretion may be due to a missing or partial signal sequence, I would have been motivated to confirm whether there was indeed more sequence information at the amino end of the molecule that was missing from the initial cDNA clone.

Paragraphs 7.2 & 8.3

7.50 In paragraph 7.2 Dr Alitalo states:

> "...it is readily apparent from the autoradiogram that the expression level of VEGF-C is much higher than that of VEGF2(HGS)".

7.51 Further, at paragraph 8.3 Dr Alitalo states:

> "The fact that VEGF-C expression observable in cell lysates of VEGF-C-transfected cells is much higher than VEGF2(HGS) expression observable in VEGF2(HGS)-transfected cells suggests that VEGF2(HGS) is inefficiently translated and/or that the intracellular turnover rate of VEGF2(HGS) is much faster than that of VEGF-C. In other words, the cells may be recognizing VEGF2(HGS) as an aberrant protein and rapidly degrading it."

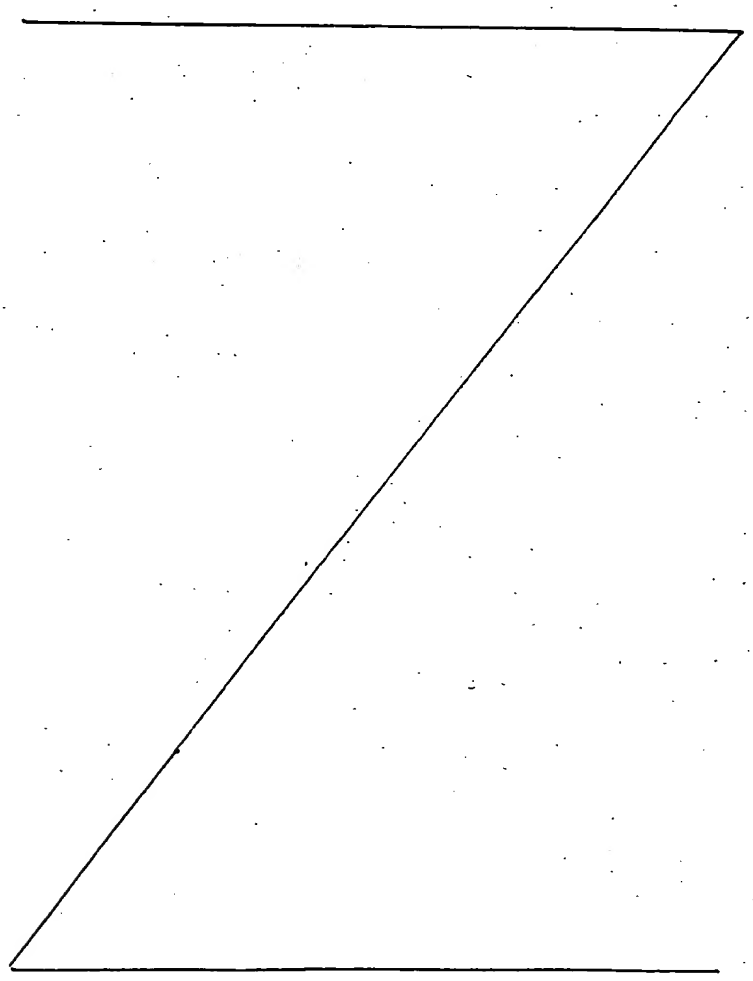
7.52 In my opinion Dr. Alitalo's conclusions represent pure speculation and cannot reasonably be drawn from the results presented in his statutory

declaration for two reasons. First, Dr. Alitalo used two different antibodies to compare the VEGF2(HGS) protein and VEGF-C. Moreover, he has not established that the detection capacity of these two antibodies is the same. Second, the position of incorporation of the HA tag into the VEGF2 construct would appear to be in a position that Dr Alitalo himself has shown to result in inefficient immunoprecipitation of active secreted VEGF C.

(ii) Dr. Ballard's Statutory Declaration

7.53 I refer to Dr. Ballards' Statutory Declaration, and make the following comments.

7.54 Dr Ballard's Statutory Declaration merely confirms the statements made in the Statutory Declarations by Associate Professor Rogers and Dr. Alitalo. I repeat my comments above in response to this Statutory Declaration



AND I make this solemn declaration by virtue of the Statutory Declarations Act, 1959 and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DATED this Twelfth day of December 2000.

DECLARED at: Geneva)

BEFORE me: Pierre CHRISTEN)

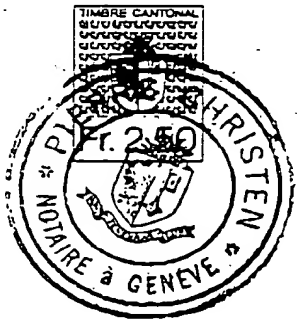
.....

JENNIFER RUTH GAMBLE

.....

Commissioner of Declarations/patent attorney/Justice of the Peace/Solicitor

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12th December 2000.-



COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

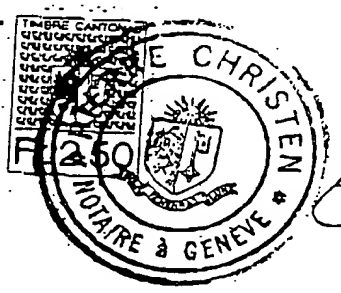
- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure JRG-1** referred to in my Statutory Declaration made this Twelfth day of December 2000.

Jennifer Ruth Gamble

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12th December 2000/mb.-



WITNESS: _____

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

CURRICULUM VITAE

NAME: GAMBLE, Jennifer Ruth

BORN: 4 September, 1952

QUALIFICATIONS:

1978 B. App. Sc. (RMIT, Victoria)
1986 M.Sc. (University of Melbourne)
1994 Ph.D. (University of Adelaide)
1998 Associate Professor (University of Adelaide)

CONTACT: Telephone: (61 8) 8222-7482
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PRESENT APPOINTMENT:

Research Scientist, Institute of Medical & Veterinary Science, Adelaide
Founding Member, Hanson Centre for Cancer Research, IMVS, Adelaide
Co-Head, Vascular Biology Unit, Hanson Centre for Cancer Research
Associate Professor, Department of Medicine, University of Adelaide

PREVIOUS APPOINTMENTS:

1971-79	Research Assistant, Experimental Pathology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne
1979-80	Visiting Research Assistant at Institute for Immunology, Marseille, France
1979-85	Research Assistant, Thymus Biology Unit, The Walter and Eliza Hall Institute of Medical Research, Melbourne
1985	Visiting Scientist, University of Washington, Seattle, USA
1985-92	Research Scientist, Division of Human Immunology, Institute of Medical and Veterinary Science, Adelaide
1991	Visiting Scientist, Scripps Research Institute, San Diego, USA.
2000-2001	Visiting Professor, University of Geneva, Switzerland

MEMBERSHIP OF PROFESSIONAL SOCIETIES:

Inaugural member of Australian Vascular Biology Society

COMMITTEES:

National Health & Medical Research Council of Australia, Regional Grants Committee	1997, 1998, 1999
National Heart Foundation, Australia, Regional Grants Committee	1997, 1998, 2000
National Heart Foundation, Australia, Fellowship Committee	1999
University of Adelaide, Division of Health Sciences Research Committee	1996-2000
Women's & Children's Hospital, Adelaide, Research Grants Committee	1996-1998

REFEREE FOR GRANTING BODIES:

National Health and Medical Research Council of Australia
National Heart Foundation. Australia
Anti-Cancer Foundation of the Universities of South Australia
Anti-Cancer Foundation of Victoria
Anti-Cancer Foundation of New South Wales
Western Australia Cancer Foundation
Arthritis Foundation of New South Wales
Arthritis Foundation of Victoria
Various University and Hospital Research Committees

REFEREE FOR JOURNALS:

Journal of Cell Biology
Journal of Clinical Investigation
Journal of Immunology
Immunology and Cell Biology
Growth Factors
Journal of Leukocyte Biology
Laboratory Investigation
Journal of Vascular Research
Atherosclerosis, Thrombosis & Vascular Biology
Thrombosis Research

THESIS EXAMINER:

University of New South Wales
University of Queensland
University of Adelaide
University of Sydney

CONFERENCE ORGANISATION:

Australian Vascular Biology Society Scientific Meeting	1994 and 1999
Hanson Centre for Cancer Research Symposium	1994, 1996, 2000

GRANTS HELD

(Previous):

Australian Brain Foundation M Vadas, G Burns, A Lopez, J Gamble
"Endothelium and white blood cell interaction of the pathogenesis of cerebrovascular disease"
1986 $5000

National Health & Medical Research Council (No. 860756) M Vadas, J Gamble
"Regulation of granulocyte endothelial interactions in man"
1986-1988 $54,750, $42,732, $42,732

Anti-Cancer Foundation of the Universities of South Australia G Burns, D Gillis, J Gamble
"Molecules mediating adhesion of tumour cells: their role in distribution and metastatic spread"
1987 $24,000

Anti-Cancer Foundation of the Universities of South Australia A Lopez, J Gamble, LB To
"Identification of a novel growth factor for human hemopoietic cells: biological and molecular characterisation" 1987 $25,000

National Heart Foundation M Vadas, J Gamble
"Role of TNF-α and IL-1 in thrombotic phenomena" 1987-1988 $37,594 & $33,069

Anti-Cancer Foundation of the Universities of South Aust A Lopez, JR Gamble, LB To
"Regulation of leukaemia cell proliferation and differentiation by recombinant human IL-3"
1988-1989 $24,000 pa

National Health & Medical Research Council (No. 980844) MA Vadas, JR Gamble
"Regulation of endothelial adhesiveness for blood cells in man"
1989-1991 $51,000 $52,000 $53,000

Anti-Cancer Foundation of the Universities of South Australia JR Gamble, MA Vadas
"*In vitro* models of angiogenesis: investigations of events involved in capillary invasion of neoplasms" 1990-1991 $32,764 $37,400

National Heart Foundation MA Vadas, JR Gamble, PJ Nestel
"Regulation of monocyte-endothelial cell interactions" 1990-1992 $33,000 pa

National Health & Medical Research Council (No. 920866) MA Vadas, JR Gamble
"Mechanism of action of proteins that inhibit neutrophil-endothelial interactions"
1992-1993 $87,320 $91,994 $91,994

National Health & Medical Research Council (No. 920867) JR Gamble, P Kaur
"Cell surface antigens involved in angiogenesis" 1992-1993 $65,217 & $66,227

Anti-Cancer Foundation of the Universities of South Australia JR Gamble, P Kaur
"Purification of novel factors involved in the process of new blood vessel formation (angiogenesis)" 1994 $41,900

Royal Adelaide Hospital "β1 antigens" JR Gamble 1994 $10,000

The Kathleen Cuningham Foundation for Breast Cancer Research JR Gamble
"Dominant mutant retinoblastoma gene product as a potential regulator of angiogenesis"
1996-1997 $30,000 pa

National Heart Foundation J Gamble and P Xia
"New Mechanism of Atheroprotection: High Density Lipoproteins, Endothelial Adhesion Molecule Expression and the Sphingomyelin Pathway" 1998-1999 $47,000 pa

Anti-Cancer Foundation of South Australia J Gamble and B Wattenberg
"Lumen Formation in Angiogenesis: Characterisation of the membrane-targetting molecules that regulate intracellular vesicle fusion as a prerequisite for lumen formation" 1999 $47,000

(Current):

National Health & Medical Research Council Program Grant
M Vadas, A Lopez, J Gamble, P Cockerill, G Goodall
"Leukocyte and Endothelial Cell Biology" 1997-2001 $928,846 pa

National Health & Medical Research Council Large Equipment Grant
J Gamble, L Ashman, D Findlay and M Vadas $22,000

National Heart Foundation P Xia and J Gamble
"A Novel Signalling Pathway in Atherosclerosis" 2000-2001 $45,000 pa

Faculty of Health Sciences, University of Adelaide J Gamble and M Vadas
Equipment Grant, 2000 $48,000

Rebecca Cooper Medical Research Foundation J Gamble
"Role of Sphingosine Kinase in Arthritis" 2000 $7,500

PATENTS

A Method of Modulating Cellular Activity. (PCT/AU98/00730) Provisional file, September 1997. Vadas MA, Gamble JR, Xia P, Barter P, Rye K-A, Wattenberg B and Pitson S.

A Novel Cation Chloride Cotransporter Molecule and genetic sequences encoding same. (PP7008/98) Provisional file, November 1998. Medvet Science Pty Ltd. Hiki K, Gamble JR, Vadas MA, D'Andrea R and Sutherland G.

Novel therapeutic molecules and uses thereof - I. Provisional Patent (PQ0339/99) 13 May 1999. Pitson SM, Wattenberg BW, Xia P, D'Andrea RJ, Gamble JR, Vadas MA. App No PQ8408/00.

Novel therapeutic molecules and uses thereof - II. Provisional Patent (Q1504/99) 8 July 1999. Pitson SM, Wattenberg BW, Xia P, D'Andrea RJ, Gamble JR, Vadas MA.
(I and II Combined: PCT/AU00/00457).

INVITED SPEAKER:

Australian Society for Medical Research, Adelaide 1991
Baker Symposium, Melbourne 1992
Annual Inflammation Symposium, Sydney 1993, 1994
Australian Vascular Biology Society Annual Meeting 1993, 1995, 1997

Hanson Centre for Cancer Research Symposium	1994, 1996
Asia Pacific League of Associations of Rheumatology Meeting, Melbourne	1996
International Vascular Biology Society Meeting, Seattle, USA	1996
Lorne Cancer Meeting	1997
International Vascular Biology Society Meeting, Cairns, Australia	1998
Keystone USA Symposium "The Endothelium"	1998
Australasian Haematology Society, Adelaide	1998

SYMPOSIUM CHAIRPERSON

Australian Vascular Biology Society Meeting	1994, 1996
International Vascular Biology Society, Seattle, USA	1996
International Vascular Biology Society, Cairns, Australia	1998

PUBLICATIONS:

1. Basten, A., Miller, J.F.A.P., Warner, N.L., Abraham, R., Chia, E. and GAMBLE, J. (1974) A subpopulation of T cells bearing Fc receptors. Journal of Immunology 115:1159-1165.

2. Miller, J.F.A.P., Vadas, M.A., Whitelaw, A. and GAMBLE, J. (1975) H-2 gene complex restricts transfer of delayed-type hypersensitivity in mice. Proceedings of the National Academy of Sciences USA 72:5095-5098.

3. Miller, J.F.A.P., Vadas, M.A., Whitelaw, A. and GAMBLE, J. (1975) A radioisotopic method to measure delayed type hypersensitivity in the mouse. II. Cell transfer studies. International Archives of Allergy and Applied Immunology 49:693-708.

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5. Basten, A., Miller, J.F.A.P., Abraham, R., GAMBLE, J. and Chia, E. (1975) A receptor for antibody on B lymphocytes. III. Relationship of the receptor to immunoglobulin and Ia determinants. International Archives of Allergy and Applied Immunology 50:309-321.

6. Miller, J.F.A.P., Vadas, M.A., Whitelaw, A. and GAMBLE, J. (1976) Role of major histocompatibility complex gene products in delayed type hypersensitivity. Proceedings of the National Academy of Sciences USA 73:2486-2490.

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24. Klebanoff, S.J., Vadas, M.A., Harlan, J.M., Sparks, L.H., GAMBLE, J.R., Agosti, J.M. and Waltersdorph, A.M. (1986) Stimulation of neutrophils by tumour necrosis factor. Journal of Immunology 136:4220-4225.

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COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure JRG-2** referred to in my Statutory Declaration made this Twelfth day of December 2000.

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12th December 2000/mb.-

Jennifer Ruth Gamble

WITNESS: _____

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

Summary Research History

Jennifer Ruth Gamble

1.1. From 1971 to 1979 I was a Research Assistant with the Experimental Pathology Unit at the Walter Eliza Hall Institute for Medical Research. During that period in time I studied the role of the histocompatibility complex in immune responses concentrating on delayed-type hypersensitivity reactions.

1.2. Between 1979 and 1980 I was a Visiting Research Assistant at the Institute for Immunology in Marseille in France. I also held the position of Research Assistant with the Thymus Biology Unit at the Walter Eliza Hall Institute for Medical Research in Melbourne. It was during this period that I was investigating the biological and biochemical nature of the T Cell Receptor.

1.3. In 1985, I took a sabbatical in Seattle at the University of Washington where I started to learn the techniques of endothelial cell isolation and began to examine endothelial cell function. In particular, I studied the adhesion of inflammatory cells such as neutrophils to the endothelium. While carrying out these studies I obtained a sample of TNF-α, which had recently been cloned at Genentech and used it in the controls in the experiments that I was conducting. From those experiments I established that endothelial cells could be regulated by inflammatory cytokines like TNF-α. This was the first description that the phenotype of endothelial cells could be altered. The results of that research were published in 1985 in publication 22 in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2a**" is a true copy of publication 22. That article is widely quoted in the area of inflammation. During and after that period I investigated the effects of a number of cytokines on the function of the endothelium and inflammatory cells. These cytokines included GM-CSF, IL4, IL5 and IL3.

1.4. Towards to the end of 1985 I moved to the Institute of Medical and Veterinary Science (IMVS) in South Australia, where I remain today. Upon arriving in Adelaide I established the Vascular Biology Unit at the IMVS. My research at that time continued to look at the regulation of endothelial cell function with particular emphasis on adhesion molecules and inflammatory type responses in endothelial cells.

1.5. Between 1985 and about 1990 I studied the regulation of adhesiveness of endothelial cells. It was during this period that I discovered that the cytokine TGF-β inhibited TNF action. That research was published in the prestigious journal *Science* in 1988 and is identified as publication 33 in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2b**" is a true copy of publication 33. Thus, I had demonstrated that adhesion molecule expression on endothelial cells could be both stimulated (eg by TNF) and inhibited (eg by TGF).

1.6. I have continued to investigate the adhesive properties of the endothelium. However about 1991, my colleagues and I started to consider the role of endothelial cells in angiogenesis. Therefore we established assays to measure endothelial cells undergo angiogenesis. An *in vitro* assay system that measured capillary tube formation, which is classically referred to as the gel-type assay was established since this measured many different aspects of angiogenesis.

1.7. The major difference between the assay that I set up and the gel-type assays that were routinely carried out by other research groups at that time was that my assay was performed in microtitre wells. This was developed as a means to screen large numbers of molecules under different biological conditions for pro-angiogenic or inhibitory functions. The assay was reported in 1993 in publication 51 identified in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2c**" is a true copy of publication 51. The same publication was also one of the first reports showing that cell surface molecules called integrins are involved in angiogenesis.

1.8. By about 1991 my laboratory was using three different assays to measure aspects of angiogenesis *in vitro*. They were the cell proliferation assay, improved gel type assay and a wound assay. In the wound assay, a monolayer of endothelial cells is damaged by way of a scrape and measurement is made of the ability of the damaged monolayer to repair itself.

1.9. By 1991 VEGF was also starting to become the focus of a great deal of scientific research. While VEGF had been postulated to exist well prior 1991, it was around 1991 that Genentech first described the cloning of the molecule.

1.10. Shortly after Genentech cloned VEGF I received some samples of the molecule to study. Since my return from the University of Washington in 1985 I had maintained my collaboration with Genentech. They knew that I had set up a number of assays for measuring endothelial activity. Hence we extended our collaboration with them to investigate the biological activity of VEGF.

1.11. When I obtained the Genentech VEGF material I began to look at various aspects of VEGF activity together with another angiogenic cytokine molecule that was also being described at that time, namely Fibroblast Growth Factor (FGF). Initially both VEGF and FGF were examined using the proliferation assay that I had set up. Those studies provided a quick and easy way to confirm that the material that I had obtained from overseas was in fact active, since both VEGF and FGF were known to be mitogens. I then set out to study the activity of these cytokines in the gel assay that I had developed. My gel assay studies examined the activity that both FGF and VEGF displayed in the angiogenic process. I also looked at the effects of FGF and VEGF on molecules such as the integrins and other signalling molecules that were known to be important in the process of angiogenesis. These studies were carried out to determine the activity of the growth factors, how they influenced other molecules and how they modulated the extent and characteristics of angiogenesis.

1.12. In addition to the abovementioned studies my colleagues and I also started, in 1993, to examine the morphology of the endothelial cells as they were starting to form capillary tubes. In 1997, my colleagues and I described, for the first time, how lumina form during angiogenesis. That research was published in publication 73 identified in my *curriculum vitae*. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2d**" is a true copy of publication 73. However, still to date little is known of the molecular mechanisms or events that are needed to reorganize endothelial cells to provide their three-dimensional structure.

1.13. While conducting our morphology studies I also initiated a small project to look for inhibitory molecules of the process of angiogenesis. That research, which I carried out between 1994 and 1996, sought to identify antibodies that bound to endothelial cells which blocked the process of angiogenesis. In that research, monoclo[illegible] antibodies were generated against the endothelial cell surface. The monoclonal antibodies were then screened

using the gel assay that I had developed, to test whether any were capable of inhibiting angiogenic activity. While we were able to produce hundreds of different monoclonal antibodies, we were not able to identify a specific antibody that was particularly attractive in its anti-angiogenic activity.

1.14. I also started to examine the regulation of cell junctions, which we postulated at that time, might be regulated by VEGF. For endothelial cells to make a new blood vessel they need to break their existing cell junctions (interactions) with their neighbours thus allowing them to migrate and proliferate. Inhibition of angiogenesis would suggest the reforming of tight junctions. Publication number 72 in my *curriculum vitae* demonstrates that junctional molecules are not only structural but also provide important signals to the endothelial cell that has consequences to the phenotype of the cell. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2e**" is a true copy of publication 72.

1.15. After my colleagues and I published our finding in publication number 72 in my *curriculum vitae* we went on to determine that VEGF165 alters these junctional molecules consistent with the hypothesis that the tightness of the junction would be decreased. We also showed that VEGFB and VEGF121 induced similar changes. Further, we also showed that no major differences were seen between the three forms of VEGF in the assays performed which included capillary tube formation in collagen gels, permeability induction and proliferation.

1.16. More recently we have determined that another "angiogenic factor" Angiopoietin1 is able to inhibit the VEGF mediated induction of permeability and changes the junctional molecules involved in permeability. Now produced and shown to me and exhibited hereto marked with the letters "**JRG-2f** "is a true copy of publication 91.

1.17. In addition to the above research we have been isolating genes involved in angiogenesis with the aims of expanding our understanding of the downstream targets of endothelial cell activation and thus ultimately defining novel therapeutic targets. That research continues today.

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

OPPOSITION THERETO BY: Ludwig Institute for Cancer Research Under Section 59 of the Patents Act.

This is **Annexure JRG-3** referred to in my Statutory Declaration made this Twelfth day of December 2000.

Sworn and subscribed to before me Pierre CHRISTEN, Notary Public in Geneva, this 12 December 2000/mb.-

Jennifer Ruth Gamble

TIMBRE CANTONAL Fr 2.50 CHRISTEN NOTAIRE à GENEVE

WITNESS: _____

Commissioner for Declarations/Solicitor
Patent Attorney/Justice of the Peace

COMMONWEALTH OF AUSTRALIA

(Patents Act 1990)

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

- and -

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

DOCUMENT LIST

Documents provided to me by the Patent Attorneys representing HGS in the subject proceedings are as follows:

1. U.S. Patent No. 6,130,071, issued October 10, 2000, to Alitalo et al.

COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF : Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Tom Rapoport of Harvard Medical School, of Harvard University, Boston, Massachusetts, United States of America, declare as follows:

1. I am currently a Professor of Cell Biology for the Howard Hughes Medical Institute in the Department of Cell Biology at the Harvard Medical School in Boston, Massachusetts. In 1995, I was appointed Professor of Cell Biology at Harvard Medical School. Prior to that appointment, I was Group Leader at the Max-Delbruck-Center for Molecular Medicine, Germany, from 1992-1994. From 1985-1992, I was the Group Leader at the Institute for Molecular Biology, Germany. From 1972-1985, I was a Research Assistant at the Zentralinstitut for Molekularbiologie der Akademie der Wissenschaften der DDR.

2. Since the early 1970s, my research has focused on protein processing and intracellular transport and the role of signal sequences in protein translocation across cell membranes, as evidenced by my curriculum vitae, which lists the publications that I have authored or co-authored. My research in the area of protein translocation has encompassed bacterial, yeast and mammalian systems. I am also broadly interested in examples of nonclassical secretion

pathways of mammalian proteins, including, fibroblast growth factor (FGF). Now shown to me and marked "TP-1" is a copy of my curriculum vitae.

3. I have been asked by the Patent Attorneys representing Human Genome Sciences ("HGS") to review Australian Patent Application Au-B-696764 (73941/94) in the name of HGS, entitled "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), which claims priority and has a virtually identical specification to U.S. application no. 08/207,550, filed March 8, 1994. I have also been asked to provide my comments and opinions as to what the patent specification would provide or teach to one of ordinary skill in the art of protein processing as of the earliest filing date of the HGS patent specification, March, 1994. For purposes of this analysis, I considered not only what I knew and appreciated at the relevant time, but what was expected to be known by graduate students and postdoctoral fellows who were in my laboratory at the relevant time. I have also been asked to comment on the state of the art of signal sequences and protein processing as of the earliest filing date of the HGS patent specification, March, 1994.

4. By the late 1970s, secreted proteins and the signal sequences required to route these proteins through the cell were well understood. For example, it was known that specific signals, often called signal sequences, were required to direct secreted proteins outside of the cell. These signal sequences were known to be located at the N-terminus of the secreted protein and removed either during or shortly after translocation of the protein across the endoplasmic reticulum and to the outside of the cell.

5. By 1994, it was known that, in mammals, signal sequences were typically located at the beginning of the secreted protein, often comprising 20 amino acids, and were characterized by a stretch of at least six or seven consecutive hydrophobic amino acids, the majority of which are leucine residues. Outside of the consecutive hydrophobic residues, there were other known requirements

for signal sequences, including the type of residues which provide the information to direct the cell to remove the signal sequence, i.e., the cleavage site. This cleavage site was known to contain small aliphatic residues, such as alanines at positions -1 and -3 to the N-terminus of the cleavage site, and was known to be usually found five to seven residues downstream from the stretch of consecutive hydrophobic residues.

6. By 1994, researchers were able to characterize signal sequences by their hydrophobic nature. Such sequences were often identified by an inspection of the amino acid sequence. Alternatively, computer programs, such as P SORT and SIGNAL P were, and still are today, standard tools used to predict the presence or absence of signal sequences. There was however no certainty with such approaches.

7. I have reviewed and analyzed the polynucleotide, and amino acid sequence, identified by HGS to encode the human VEGF-2 protein, as set forth in Figure 1 of the HGS patent specification. The human VEGF-2 protein is described in the HGS patent specification as structurally related to the PDGF/VEGF family of growth factors, a known family of secreted proteins. The HGS patent specification further states that the VEGF-2 polynucleotide is predicted to contain an open reading frame of approximately 350 residues, which encodes VEGF-2. (See, the HGS patent specification at page 5, lines 25-27). The specification reports that at the amino acid level VEGF-2 exhibits the highest homology to vascular endothelial growth factor (30% identity), followed by PDGF alpha (23%) and PDGF beta (22%). (See, the HGS patent specification at page 5, lines 28-31). The VEGF-2 protein was further characterized in the HGS patent specification as containing eight cysteines which are conserved among all four members of the family, and in addition also contains the conserved or signature motif PXCVXXXRCXGCCN which is found in all members of the PDGF/VEGF family. (See, the HGS patent specification at page 5, lines 31-33). The HGS patent specification speculates that the first 24

residues of the 350 amino acid sequence may encode a signal sequence. (See, the HGS patent specification at page 5, lines 26-27).

8. Attached as "Annexure TP-2" is an annotated copy of Figure 1 of the HGS patent specification which sets forth the nucleotide and amino acid sequence of VEGF-2 identified by HGS. The figure is further annotated to indicate specific amino acid residues and those portions of the VEGF-2 sequence which will become relevant throughout this declaration. For consistency of nomenclature, the numbering of amino acid residues will be referred to in the context of what is now known as the 419 amino acid form of VEGF-2. As shown in Annexure TP-2, the 350 amino acid form of VEGF-2 corresponds to amino acid residues 70 to 419 of the 419 form of VEGF-2.

9. By 1994, had I or any one skilled in the art, such as any Ph.D. scientist or candidate in my laboratory, been presented with the HGS patent specification, (see ¶ 7 above) one would recognize that the VEGF-2 protein is a secreted protein. Based on the characterization of the VEGF-2 protein set forth in the HGS patent specification, one would recognize that the protein was a member of the PDGF/VEGF family of growth factors. The PDGF/VEGF family of growth factors, like other growth factors, must be secreted in order to exert their growth promoting or mitogenic effects. Since all previously identified members of the PDGF/VEGF family were known to be secreted, one would expect the newly identified VEGF-2 to also be secreted.

10. Had I or any skilled artisan been presented with the HGS patent specification in 1994 and wanted to express the VEGF-2 protein, one would proceed with inspecting the 350 amino acid sequence set forth in Figure 1 to identify a potential signal sequence. As I have already noted, by 1994, this was routinely achieved by visual inspection or could be achieved with the aid of a computer program. Upon inspection of the N-terminal portion of the 350 amino acid sequence, I did not observe the typical conserved motif of a signal sequence.

In fact, the N-terminal portion of the 350 amino acid sequence is not very hydrophobic and contains many charged residues. However, provided with the strong evidence that the 350 amino acid sequence was a secreted protein based on the teaching and recognition of the HGS patent specification that it is a member of a family of secreted growth factors (see ¶ 7 above), I would not rule out that the 350 amino acid sequence may contain an atypical signal sequence. Atypical signal sequences are not without precedent. For example, as of March 1994, it was known that ovalbumin, a secreted protein, contains an atypical and uncleaved signal sequence that is not immediately obvious on simple inspection.

11. The possibility of additional upstream coding regions from the sequence disclosed in Figure 1 of the HGS patent specification would not have dissuaded me nor do I believe it would have dissuaded my Ph.D. students or post-doctorate students from attempting to express the 350 amino acid sequence. By 1994, it was well established that the PDGF/VEGF family of growth factors were expressed initially as precursor proteins which underwent proteolytic processing resulting in a mature form of the protein. Thus, I would predict VEGF-2 to be expressed in a similar way. The 350 amino acid sequence set forth in Figure 1 contains those conserved motifs which are signature motifs for an active form of the protein belonging to the PDGF/VEGF family. Thus in 1994, I would have predicted the protein encoded by the sequence disclosed in Figure 1, containing motifs characteristic of the PDGF/VEGF family, to be secreted and biologically active.

12. By 1994, in order to ensure secretion of the VEGF-2 sequence disclosed in Figure 1, I would have engineered a heterologous signal sequence upstream from the methionine at position 70. Indeed, this approach is specifically taught in the HGS patent specification (at page 14, lines 6-23). Moreover, it would have involved routine practice in 1994 to select a strong signal sequence, such as that of human growth factor or insulin growth factor, and to

engineer such a construct to achieve expression and secretion of the gene product. Even given the possibility that the 350 amino acid sequence may contain an atypical signal sequence, I would still have utilized a strong signal sequence to ensure efficient secretion of the protein, and it would have been standard practice to do so. The upstream signal sequence would be expected to override any weaker signal sequence that may be present downstream, and none of the segments of amino acids in the 350 amino acid sequence are hydrophobic enough to prevent the secretion of the protein through the endoplasmic reticulum and to the outside of the cell. Thus, I would have fully expected to achieve expression and secretion of the VEGF-2 protein using a heterologous signal sequence.

13. The expectation that engineering a signal sequence upstream of the sequence set forth in Figure 1 would result in the expression and secretion of a biologically active protein as set forth in the HGS patent specification, has in fact been subsequently confirmed by one skilled in the field of VEGF-2, Dr. Kari Alitalo. Drs. Kari Alitalo and Vladimir Joukov are named as co-inventors of U.S. Patent No. 6,130,071, issued October 10, 2000, entitled: "Vascular Endothelial Growth Factor C (VEGF-C) Cys 156 Protein and Uses Thereof" (the "Alitalo Patent"). It is my understanding that VEGF-2 and VEGF-C are terms used to refer to the same molecule. The Alitalo Patent describes several fragments of VEGF-C which were engineered to be expressed and secreted from cells. Cells were engineered to express the VEGF-2 fragments by fusing a signal sequence to the nucleotide sequence encoding the protein fragment. Secreted protein fragments were obtained from the cell culture medium and tested for activity. The working examples of the Alitalo Patent demonstrate that VEGF-C fragments spanning residues 103-419 or 112-419 (see Annexure TP-2) which are fused in frame to a signal sequence are secreted into the culture medium. (Alitalo Patent, column 47, lines 44 to 48). The Alitalo Patent also demonstrates the use of a heterologous signal sequence to ensure secretion of a fragment of VEGF-C. A fragment

spanning residues 104-213 (see Annexure TP-2) was fused in frame to a heterologous signal sequence which resulted in the secretion of a biologically active form of VEGF-C. (Alitalo Patent, column 46, lines 5 to 10). Furthermore, a publication subsequent to the priority date of the HGS patent specification, which Dr. Alitalo co-authored, Joukov et al, 1997, EMBO J. 16:3898-3911 ("Joukov"), describes two VEGF-C mutants in which the native VEGF-C signal sequence was fused in frame with residues 103-419 or residues 103-227. Both VEGF-C mutants were shown to be secreted into the culture medium, (Joukov at page 3901) using experimental procedures that were routine by 1994. Thus, both Alitalo and Joukov confirm that the fusion of a signal sequence in frame to VEGF-2 fragments will result in secreted protein fragments.

14. The expectation that I would have had from reading the HGS patent specification and the expectation that I would have expected others to have had is that the sequence set forth in Figure 1 does indeed contain the conserved motifs which would confer biological activity to the VEGF-2 protein as set forth in the HGS patent specification (see ¶ 7 above) has also been subsequently confirmed by Dr. Alitalo. In the Alitalo Patent and Joukov, the VEGF-2 protein fragments were tested for VEGF-C biological activity in different assays. The Alitalo Patent expressed and assayed several fragments in this manner, including fragments spanning residues 103-225, 103-419, 104-213, and 112-419 (see Annexure TP-2), and as described in ¶ 13, above. Each of the fragments assayed were found to be biologically active. Based on these observations and sequence comparisons of the VEGF family, the Alitalo Patent states that still smaller fragments of the 419 amino acid sequence of VEGF-C will retain biological activity. (Alitalo Patent, column 47, line 57 to column 48, line 2). The Alitalo Patent further states that a protein which retains the conserved motif RCXXCC (e.g., a polypeptide comprising from about residue 161 to about residue 211, see Annexure TP-2) is postulated to

retain biological activity. (Alitalo Patent, column 47, line 57 to column 48, line 2).

15. As observed in the Alitalo Patent and Joukov, VEGF-C fragments spanning residues 103-225, 103-419, 104-213, and 112-419 (see Annexure TP-2) fused in frame with signal sequences contained sufficient information to be processed by the cell to result in a biologically active form of VEGF-C. Furthermore, Alitalo also predicts that a VEGF-C fragment spanning residues 161-211 of VEGF-C (see Annexure TP-2) contains sufficient information to confer biological activity. Thus, if these VEGF-C fragments spanning residues 103-225, 103-419, 104-213, 112-419 and 161-211 contain sufficient information to allow for processing of a biologically active form of VEGF-C, the longer sequence set forth in the HGS patent specification which spans residues 70-419 (see Annexure TP-2), fused in frame to a signal sequence, should also contain sufficient information to be processed by the cell to result in a biologically active form of VEGF-C. According to the Alitalo Patent and Joukov, proteolytic processing of VEGF-C appears to differ depending on the cell used to achieve expression of the VEGF-C protein (Joukov at ¶ spanning pages 3906-3907). However, both Alitalo and Joukov demonstrate that regardless what type of mammalian or yeast cells are used to express VEGF-C, a biologically active VEGF-C protein is obtained. (Joukov at ¶ spanning pages 3906-3907; Alitalo Patent at column 46, lines 5-10). Consequently, regardless of the cell line used to express VEGF-C and the exact proteolytic processing or glycosylation that results, a biologically active fragment of VEGF-C can still be obtained. Thus, consistent with the teaching of the HGS patent specification, residues 70-419 as set forth in Figure 1 also contain sufficient information to confer VEGF-C biological activity.

16. The Alitalo Patent and Joukov have clearly confirmed that the teaching of the HGS patent specification, that the fusion of a signal sequence to a fragment of VEGF-C will result in the successful secretion of that fragment from the cell

into the culture medium. Thus, according to the teaching of the HGS patent specification, residues 70-419 as set forth in Figure 1 fused in frame to a signal sequence will result in its successful secretion from the cell into the culture medium.

17. In sum, as described in the HGS patent specification, Dr. Alitalo has subsequently demonstrated and/or predicted that VEGF-C fragments spanning residues 103-227, 103-419, 104-213, 112-419 and 161-211 (see Annexure TP-2) when fused in frame to a signal sequence result in a secreted gene product which retains VEGF-C biological activity. Thus, the sequence set forth in the HGS patent specification, spanning residues 70 to 419 of VEGF-C (see Annexure TP-2) fused in frame to a signal sequence should also result in a protein which is secreted and retains VEGF-C biological activity.

18. In my opinion, I or one skilled in the art would identify the VEGF-2 protein as a novel member of the PDGF/VEGF family of growth factors, and as such, would recognize that VEGF-2 is also a secreted growth factor, based on the HGS patent specification in combination with the state of the art as of March, 1994. I or one skilled in the art would recognize the utility in using a heterologous signal sequence to achieve expression and secretion of the VEGF-2 protein, based on the HGS patent specification in combination with the state of the art as of March, 1994. Hence, I or one skilled in the art following the teaching of the HGS patent specification coupled with the knowledge of the art at March 1994, would have predicted and expected to achieve expression and secretion of a protein which retains VEGF-2 biological activity and it would have been obvious to carry out those experiments to achieve that purpose.

AND I declare further that all statements made in this Declaration of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Tom Rapoport, _____.

at Boston Massachusetts, on this 13th day of ~~November~~ December 2000;

before me: _____.
Notary Public

LORNA L. FARGO
MY COMM EXPIRES 12-01-06

Curriculum vitae

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Personal data: born 06/17/47 in Cincinnati (USA)
married, 3 children

Education:

1965 - 1966	High school specialized in mathematics and natural sciences at the Humboldt-University Berlin, graduation with "honors"
1966 - 1972	Study of chemistry and biochemistry at the Humboldt-University Berlin, graduation with "honors"
1972	Ph.D. for work on "The mechanism of the inorganic pyrophosphatase of baker's yeast"
1977	"Habilitation" for work on "The development of a control theory for the mathematical modelling of metabolic pathways"

Areas of research: 1969 - 1972 enzyme kinetics, enzymology, protein purifications

	1970	- 1980	mathematical modelling of metabolic systems, studies on the regulation of the glycolysis in erythrocytes
	1972	- 1980	biosynthesis of carp insulin, cloning of the cDNA for carp insulin, expression of the gene in E.coli, oocyte injection of mRNAs
	1972	-	intracellular protein transport, transport of proteins across the endoplasmic reticulum membrane, membrane biogenesis
Academic positions:	1972	- 1985	Research Assistant at the Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften der DDR
	1985	- 1992	Professor of Cell Biology
	1985	- 1992	Group Leader at the Institute for Molecular Biology
	1992	- 1994	Group Leader at the Max-Delbrück-Center for Molecular Medicine
	1995	- 1997	Professor of Cell Biology at Harvard Medical School
	1997	- present	HHMI Professor of Cell Biology at Harvard Medical School

Memberships: German Biochemical Society
Academy of Sciences
Academea European
EMBO
American Society for Cell Biology

Honors: Johannes-Müller-prize of the Society for Experimental Medicine

Rudolf-Virchow-prize

Sabbatical:	1982	3 month stay in the laboratory of Dr. G. Blobel (Rockefeller University New York)

Major Committee Assignments:

1996-1997	Standing Committee on Promotions, Reappointments and Appointments in the Faculty of Medicine at Harvard Medical School
1996-present	Various promotion committees
1996-present	Various Ph.D. Thesis Committees
1997-1999	Vice Chair/Chair, Gordon Research Conference on Molecular Membrane Biology
1999-present	NIH study section (permanent member)

Editorial Boards:

1980-1988	FEBS Letters
1989-present	EMBO Journal
1989-present	The Journal of Cell Biology
2000	EMBO Reports

Teaching Report:

1996-1998	Co-director, Cell Biology 201 course
1999-2000	Director, Cell Biology 201 course
1998-1999	CBC course (medical students)
1998-1999	Molecular Machines course

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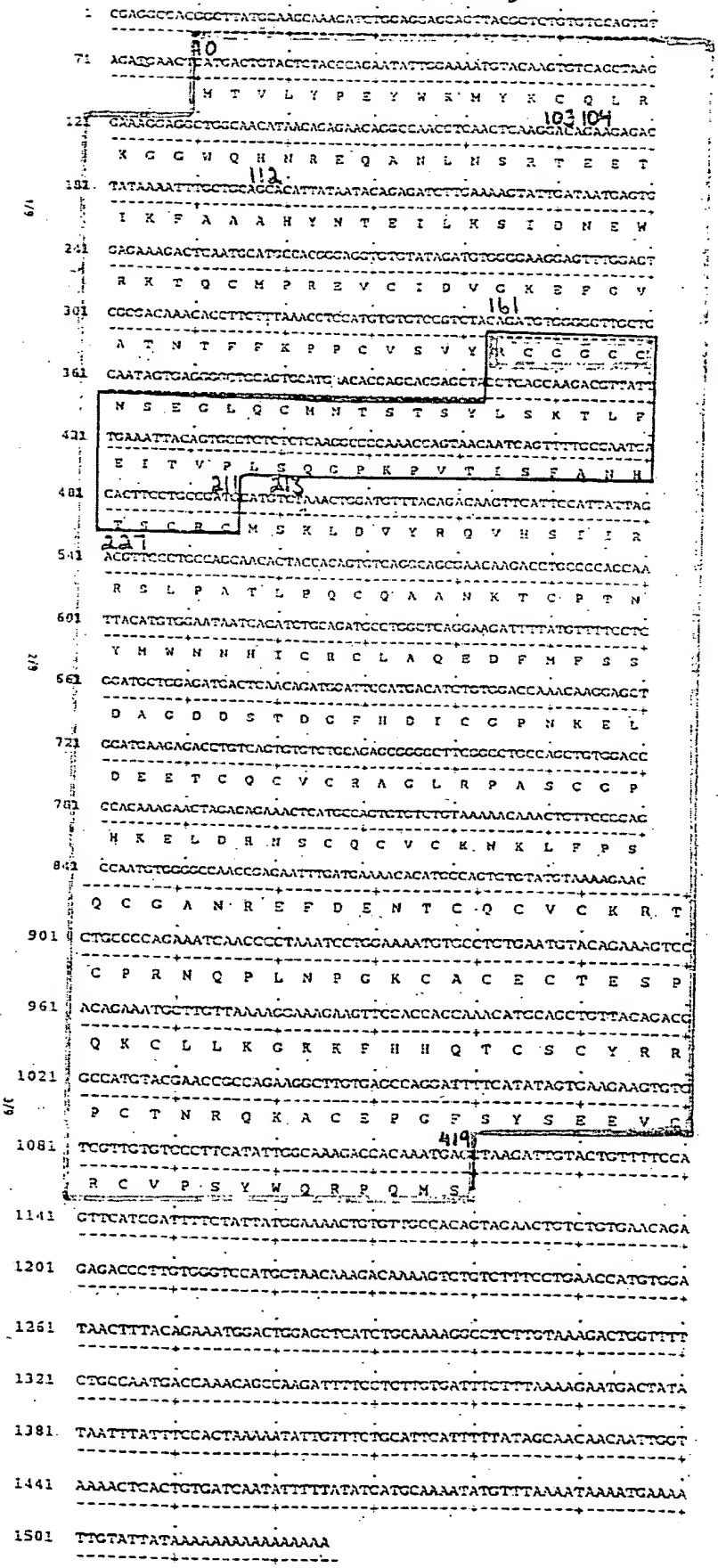
FIG. 1A-D



HGS VEGF-2 350 amino acid sequence (70-419)

Minimum sequence required to maintain VEGF-C biological activity as identified by Alitalo

RCXXCC conserved motif

Alitalo VEGF-C fragments:
103-227
103-419
104-213
112-419

ANNEXURE TP-2

COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF : Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Stuart A. Aaronson of Mount Sinai Medical Center, New York, New York, United States of America, declare as follows:

1. I am currently the Director of the Dorald H. Ruttenberg Cancer Center for the Mount Sinai Medical Center in New York, New York. I have held this position since 1993. From 1977 to 1993, I was the Chief of the Laboratory of Cellular and Molecular Biology at the National Cancer Institute, Bethesda, Maryland. From 1970 to 1977, I was the Head of the Molecular Biology Section, from 1969 to1970, a Senior Staff Fellow and from 1967 to 1969, a Staff Associate at the Viral Carcinogenesis Branch at the National Cancer Institute. I was awarded my M.D. in 1966 from the University of California Medical School, San Francisco.

2. Since the 1970s, my research has focused on growth factors and their role in tumorigenesis and cancer as evidenced by my curriculum vitae, which lists the publications that I have authored or co-authored. My research in the area of the molecular biology of growth factors and their receptors, including keratinocyte growth factor, fibroblast growth factor and vascular endothelial growth factor, has encompassed mammalian models of tumorigenesis,

including human tumor model systems. Now shown to me and marked "Annexure 1" is a copy of my curriculum vitae.

3. I have been asked by the Patent Attorneys representing Human Genome Sciences ("HGS") to review Australian Patent Application Au-B-696764 (73941/94) in the name of HGS, entitled "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), which claims priority and has a virtually identical specification to U.S. application no. 08/207,550, filed March 8, 1994. I have been asked to review and comment on the experimental evidence provided in Dr. Alitalo's Statutory Declaration. I have also been asked to review and comment on the experimental evidence provided in a draft of Dr. Susan Power's Statutory Declaration, which the Patent Attorneys representing HGS have stated she will be serving in these proceedings. I have also been asked to provide my comments and opinions as to what the patent specification would provide to one of ordinary skill in the art of the molecular biology of growth factors as of the earliest filing date of the HGS patent specification, March 1994. For purposes of this analysis, I considered not only what I knew and appreciated at the relevant time, but what was expected to be known by graduate students and postdoctoral fellows who were in my laboratory at the relevant time.

Specific Comments Concerning The Patent Specification

4. I have reviewed and analyzed the polynucleotide, and amino acid sequence, identified by HGS to encode the human VEGF-2 protein, as set forth in Figure 1 of the HGS patent specification. The HGS patent specification describes, but is not limited to, the characterization of the VEGF-2 sequence and encoded protein. The HGS patent specification describes the human VEGF-2 protein as structurally related to the PDGF/VEGF family, a known family of secreted growth factors. The HGS patent specification further discloses that the VEGF-2 polynucleotide is predicted to contain an open reading frame of

approximately 1050 residues, which encodes VEGF-2. (See, the HGS patent specification at page 5, lines 25-27). The specification reports that at the amino acid level, VEGF-2 exhibits the highest homology to vascular endothelial growth factor (30% identity), followed by PDGF alpha (23%) and PDGF beta (22%). (See, the HGS patent specification at page 5, lines 28-31). The HGS patent specification further characterizes the VEGF-2 protein as containing eight cysteines which are conserved among all known members of the PDGF/VEGF family, and in addition, also contains the fourteen amino acid signature motif, PXCVXXXRCXGCCN, found in all members of the PDGF/VEGF family. (See, the HGS patent specification at page 5, lines 31-33). The HGS patent specification speculates that the first 24 residues of the 350 amino acid sequence may encode a signal sequence. (See, the HGS patent specification at page 4, lines 29-31, and page 5, lines 26-27).

5. Based on the characterization of the VEGF-2 protein set forth in the HGS patent specification, one would recognize that the protein was a member of the PDGF/VEGF family of growth factors. The PDGF/VEGF family of growth factors, like other growth factors, must be secreted in order to exert their growth promoting or mitogenic effects. Since all previously identified members of the PDGF/VEGF family were known to be secreted, one would expect the newly identified VEGF-2 to also be secreted.

6. By March 1994 it was well known to me and, I believe to my colleagues in the angiogenic field that the PDGF/VEGF family of growth factors were expressed initially as precursor proteins which underwent proteolytic processing resulting in a mature, secreted form of the protein. Thus, I would have predicted that VEGF-2 would be expressed in a similar way. The 350 amino acid sequence set forth in Figure 1 of the HGS patent specification contains the conserved, signature motifs for an active form of a protein belonging to the PDGF/VEGF family. Thus in March 1994, I would have predicted the protein encoded by the sequence disclosed in Figure 1 of the

HGS patent specification, containing those signature motifs characteristic of the PDGF/VEGF family to be biologically active.

Specific Comments on Experimental Evidence Supplied In Dr. Alitalo's Declaration

7. Dr. Alitalo's Declaration describes experiments designed to determine whether the 350 amino acid sequence disclosed in the HGS patent specification is secreted as a mature form of the VEGF-2 protein. These experiments utilize constructs encoding amino acids 70 to 419 of the full length VEGF-2 (i.e., amino acids 1 to 350 described in the HGS patent specification) modified to contain a hemagglutinin peptide tag (HA) fused to its carboxy terminus or a vector encoding the complete 419 amino acid sequence of VEGF-C.

8. These vectors were transiently transfected into a mammalian cell line, 293T cells. The cell lysates and culture medium were assayed for the presence of newly synthesized VEGF-2/VEGF-C proteins. VEGF-2/VEGF-C were partially purified from the cell medium and cell lysates using an immunoprecipitation procedure using antibodies. The antibody used to detect VEGF-2 (70-419) was a monoclonal antibody that recognizes the hemagglutinin peptide tag. By contrast, the antibody used to immunoprecipitate VEGF-C (1 419) was a polyclonal antibody which recognizes residues 31 to 51 of the 350 amino acid VEGF-2 polypeptide. At the outset, I note that serious flaws are introduced into Dr. Alitalo's experimental design given that two different antibodies were used in the study.

9. First, Dr. Alitalo has reported the inability to isolate VEGF-C using an antibody which recognizes the C-terminal residues 372-394 or a tag attached to the C-terminus of VEGF-2 (Joukov et al., 1997, EMBO J 16: 3898, at 3900, "Joukov"). As Dr. Alitalo has reported, the amino and carboxy terminal propeptides of the precursor form of VEGF-C/VEGF-2 undergo extensive proteolytic processing, resulting in the mature form of the protein (see, Joukov

at pages 3906-7). Dr. Alitalo has also reported that the carboxy terminal propeptide "is cleaved additionally at its C-terminus," accounting for his inability to isolate VEGF-C either by using an antiserum against the C-terminal amino acid residues 372-394 or by using a tag at the C-terminus (see Joukov at page 3900, second column). If VEGF-2 is efficiently proteolytically processed to the mature form, the tag is cleaved from the carboxy terminus. The tag no longer linked to the mature form of VEGF-2 does not allow for the isolation and detection of the mature protein. Thus, the mature secreted form would not be detected using an antibody which recognizes a tag attached to the carboxy terminus, as reflected by Dr. Alitalo's own publications.

10. The HGS scientists have reported the successful isolation of a modified VEGF-2 protein containing an HA-tag at its carboxy terminus using a monoclonal antibody to HA (See, HGS Australian Patent No. 714484 and Hu J.S. et al. FASEB J. 11 (6): 498-504). However, the HGS studies were conducted in COS cells, whereas Dr. Alitalo's experiments were conducted in 293T cells (see Dr. Alitalo's Declaration at ¶ 6.2). The significance of the different cell types used is provided by Dr. Alitalo's own publications (Joukov). This publication compares the proteolytic processing of VEGF-2 expressed by a number of different cell lines, including COS cells, PC-3 cells, HT 1080 cells, and 293 EBNA cells. The results of this comparison, as reported by Dr. Alitalo was that "[t]he proteolytic processing of the VEGF-C precursor in COS cells was less efficient when compared with other cell types." (Joukov, at page 3901, second column). Thus, as the VEGF-C precursor is processed less efficiently in COS cells, the cleavage of the HA tag from the carboxy terminus should also be less efficient, which may account for HGS's ability to successfully isolate the protein from COS cells using a carboxy HA tag.

11. Second, the use of two antibodies introduces another serious flaw into the experimental design, which in the absence of the appropriate controls prevents

drawing meaningful conclusions from the data obtained. Since the antibodies recognize completely different determinants -- one within VEGF C and the other an HA-peptide tag -- each will have different affinities for the proteins they bind. Furthermore, one antibody is polyclonal and the other is monoclonal. Consequently, it is not possible to make any quantitative comparison between the results obtained for VEGF-C and VEGF-2, since the differing efficiency with which the antibodies bind their targets does not allow direct comparison of the level of proteins present in the assayed samples.

12. The aim of Dr. Alitalo's study is to determine whether VEGF-2 (HGS) is processed correctly and secreted by a mammalian cell line. However, Dr. Alitalo's experimental design allows for the detection of both the precursor and mature processed forms of VEGF-C with an anti-VEGF-C antibody; whereas the antibody used to detect VEGF-2 recognizes an HA tag fused to the C-terminus of the unprocessed form of VEGF-2 (see, Exhibit 3 of Dr. Alitalo's Declaration). As discussed in ¶ 9 above, efficient processing of the precursor protein results in extensive processing of both the amino and carboxy terminal ends thus, preventing detection of the mature protein with a carboxy terminal tag. However, the precursor form of VEGF-2 which still retains the HA tag is readily detectable (see Exhibit 3 of Dr. Alitalo's Declaration). Thus, Dr. Alitalo's experimental design allows for the detection of both unprocessed precursor form and mature VEGF-C, whereas only the unprocessed precursor form of VEGF-2 can be detected.

13. Therefore, it is not possible to draw any meaningful conclusions about the relative efficiency of secretion of VEGF-C and VEGF-2 (70 to 419) from these data -- the same antibody should have been used in both cases. Furthermore, no controls have been carried out to determine the transfection efficiency of the plasmids used -- thus preventing any valid quantitative comparisons being drawn from the data obtained.

14. Dr. Alitalo states that the expression level of VEGF-C is much higher than that of VEGF-2 (HGS), and this may be due to inefficient translation and/or that the intracellular turnover rate of VEGF2 (HGS) is much faster than that of VEGF-C (Dr. Alitalo's Declaration at ¶ 8.3). Neither of these conclusions are supported by the data set forth in Dr. Alitalo's Declaration. As discussed in ¶¶ 9 to 12 above, it is not possible to draw any conclusions from these data based on flawed quantitative comparisons. The conclusions set forth in Dr. Alitalo's Declaration are mere speculation that are unsupported by the results obtained.

Specific Comments on Experimental Evidence Supplied in Dr. Power's Declaration

15. Thus, the question of whether the 350 amino acid sequence as set forth in the HGS patent specification does indeed contain sufficient information to result in the mature processed form of VEGF-2 when secreted from a cell has, in my opinion, been addressed and affirmatively confirmed in the experiments reported in Dr. Susan Power's Statutory Declaration.

16. By March 1994, had I found that the VEGF-2 350 amino acid sequence set forth in the HGS patent specification was not secreted, in order to ensure secretion of VEGF-2, I would have engineered a heterologous signal sequence upstream and in frame with the 350 amino acid sequence, and it would have involved routine practice to do so. Indeed, this approach is specifically taught in the HGS patent specification (at page 14, lines 6-23).

17. The expectation that I and, I believe others of ordinary skill in the field would have had, that engineering a signal sequence upstream of the sequence set forth in Figure 1 of the patent specification would result in the expression and secretion of a biologically active protein as set forth in the HGS patent specification, has indeed been confirmed by the experimental evidence provided in Dr. Power's Declaration. The experiment set forth in Dr. Power's

Declaration describes the use of two constructs, the 350 amino acid sequence of VEGF-2 (as set forth in Figure 1 of the patent specification) fused in frame with a heterologous signal sequence and the 419 amino acid sequence of VEGF-2. These two constructs were used to transform a mammalian cell line. The cells were cultured under conditions to allow the cells to express the gene products encoded by the vectors. At various time points the cell lysates and culture medium were collected and each was assayed for the presence of VEGF-2. The presence of VEGF-2 was determined by a Western blot analysis using according to Dr. Power's Declaration, a polyclonal antibody to VEGF-2 that recognizes both the unprocessed precursor form as well as the processed, secreted form of VEGF-2 (See ¶ 13 of Dr. Power's Declaration).

18. Further, in my opinion, the flaws in Dr. Alitalo's experimental design which prevented any meaningful conclusions being drawn from the data presented, have been addressed in the experiments reported in Dr. Power's Declaration. First, the same antibody was used to detect the 350 amino acid sequence and the 419 amino acid sequence. Furthermore, the anti-VEGF-2 antibody used in the study is capable of detecting both the precursor form and the mature, processed form of VEGF-2. Second, the experimental protocol included comparing transfection efficiencies, and also included positive and negative controls for the expression of VEGF-2 (i.e., the expression vector encoding full-length VEGF-2 and the expression vector in the absence of any VEGF 2 sequence). In addition, the experimental protocol allowed for detection of VEGF-2 protein expression and secretion over a 72 hour time period.

19. The results of the experiments described in Dr. Power's Declaration clearly demonstrate that the 350 amino acid sequence of VEGF-2 fused in frame with a heterologous signal sequence results in the secretion of VEGF-2 from the cell (see Figure 1 of Dr. Power's Declaration). The secreted product resulting from both the 350 signal sequence construct and the 419 amino acid sequence construct, resolves as a broad band of approximately 30 kDa, and at the later

time points, e.g., 72 hours, one can also detect, in addition to this band, a minor band of approximately 21 kDa secreted by both constructs (see Figure 1 of Dr. Power's Declaration, in particular at Gel 3, lanes 22 and 24). The observation of a broad band at approximately 30 kDa and a minor band at approximately 21 kDa is consistent with Dr. Alitalo's observation that the majority of secreted VEGF-C is detected as a broad doublet band of approximately 29-31 kDa and another minor band of about 21 kDa (see, Alitalo Declaration at ¶ 7.2). Dr. Alitalo's publications also confirm that a doublet at approximately 30 kDa and another band at 21 kDa reflects a correctly processed form of VEGF-2 (see Joukov at page 3898). Thus, the results present in Dr. Power's Declaration confirm that engineering a heterologous signal sequence in frame with the coding sequence of the 350 amino acid sequence set forth in Figure 1 of the patent specification, not only results in the secretion of the protein into the culture medium, but also results in the secretion of a correctly processed mature form of VEGF-2.

20. The results of the experiments reported in Dr. Power's Declaration also demonstrate that the expression of the 350 amino acid sequence of VEGF-2 with a signal sequence contains sufficient information to allow for the correct processing of the protein to a mature biologically active protein. The expression of the 350 amino acid form of VEGF-2 results in the secretion of a proteolytically processed protein which is the same size as the secreted processed form resulting from the expression of the 419 amino acid construct. The secreted proteins which result from the expression of the 419 amino acid form of VEGF-2 and the 350 amino acid form of VEGF-2 with a signal sequence are indistinguishable in size. Both are secreted as a protein which resolves as a band at approximately 30 kDa, with another minor band detectable at approximately 21 kDa (see Figure 1 of Dr. Power's Declaration, at Gel 3, lanes 22 and 24). This is in agreement with Dr. Alitalo's observations of the mature biologically active form of VEGF-C as set forth in his declaration and publication (see ¶ 19 above). Thus, consistent with the

teaching of the HGS patent specification, the 350 amino acid sequence with a signal sequence, as set forth in the patent specification, contains sufficient information to result in the correct proteolytic processing of the VEGF-2 protein.

21. The expectation that the sequence set forth in Figure 1 of the patent specification does indeed contain the conserved motifs which would confer biological activity to the secreted VEGF-2 protein has also been confirmed by the experimental evidence provided by Dr. Power's Declaration. The results presented in Dr. Power's Declaration clearly demonstrate the 350 amino acid sequence of VEGF-2 fused in frame with a signal sequence results in the secretion of a proteolytically processed form which is resolved as a band at approximately 30 kDa and another minor band at approximately 21 kDa, the same species observed with expression of the 419 amino acid form of VEGF-2 (see Dr. Power's Declaration, Figure 1 at Gel 3, lines 22 and 24). The 30 kDa and the 21 kDa species have been consistently identified in the art as the correct processed form of VEGF-2 with biological activity. Dr. Alitalo identifies this same species as the mature biologically active form in his own declaration (see, Dr. Alitalo's Declaration at ¶ 7.2). Dr. Alitalo also identifies this same species as the mature biologically active form of VEGF-2 in his publications (see, Joukov at page 3900). Thus, the secreted VEGF-2 protein resulting from the expression of the 350 amino acid sequence set forth in the patent specification, would also be expected to have biological activity.

22. In sum, the experimental evidence provided in Dr. Power's Declaration demonstrates that the 350 amino acid sequence disclosed in the HGS patent specification fused in frame with a signal sequence results in a secreted form of VEGF-2; and that the 350 amino acid sequence contains sufficient information to be correctly processed by the cell. Furthermore, the expression of the 350 amino acid sequence with a signal sequence results in the secretion of a mature form of VEGF-2, which is indistinguishable from that observed

with expression of the 419 amino acid form of VEGF-2 as shown by both Drs. Power and Alitalo.

Conclusion

23. In my opinion, I or one skilled in the art would identify the VEGF-2 protein as a novel member of the PDGF/VEGF family of growth factors, and as such, would recognize that VEGF-2 is also a growth factor, based on the HGS patent specification in combination with the state of the art as of March 1994. I or one skilled in the art following the teaching of the HGS patent specification coupled with the state of the art, would predict that the 350 amino acid sequence with a signal sequence would result in the expression and secretion of a protein which retains VEGF-2 biological activity.

24. The experimental evidence provided in Dr. Power's Declaration confirms the teachings of the HGS patent specification, demonstrating that the 350 amino acid sequence set forth in the patent specification fused in frame to a signal sequence results in a secreted form of VEGF-2, which is correctly processed by the cell. Furthermore, these results confirm that the 350 amino acid sequence with a signal sequence contains sufficient information to be correctly processed by the cell resulting in a secreted, biologically active VEGF-2 protein.

AND I declare further that all statements made in this Declaration of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Stuart A. Aaronson, Stuart A Aaronson

at New York, New York, on this 14th day of December 2000;

before me: Maryann White

Notary Public

MARYANN WHITE
NOTARY PUBLIC, State of New York
No. 4883761
Qualified in Nassau County
Certification Filed in New York County
Commission Expires January 26, 2001

6/28/00

CURRICULUM VITAE

Name: Stuart A. Aaronson

Date and Place of Birth: February 28, 1942, Mt. Clemens, Michigan

Citizenship: U.S.A.

Marital Status: Married, three children

Education and Training:

1959-1962	B.S. (Chemistry; summa cum laude), University of California, Berkeley
1962-1966	M.D., University of California Medical School, San Francisco
1965-1966	Fellowship, Dept. of Biochemistry, University of Cambridge, Cambridge, United Kingdom
1966-1967	Intern, Medicine, Moffitt Hospital, San Francisco

Brief Chronology of Employment:

1967-1969	Staff Associate, Viral Carcinogenesis Branch, National Cancer Institute, Bethesda, MD
1969-1970	Senior Staff Fellow, Viral Carcinogenesis Branch
1970-1977	Head, Molecular Biology Section, Viral Carcinogenesis Branch
1977-1993	Chief, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland
1993-	Director, Ruttenberg Cancer Center, Mount Sinai Medical Center, New York, NY & Jane B. and Jack R. Aron, Professor of Neoplastic Diseases

Medical Licenses

New York
Virginia

Honors and Awards:

1962	Phi Beta Kappa
1966	Alpha Omega Alpha
1982	Rhoads Memorial Award
1982	PHS Meritorious Service Medal
1989	Paul Ehrlich Award
1989	PHS Distinguished Service Medal
1990	Milken Award
1991	Chirone Prize
1991	Harvey Lecture

1991 Wadsworth Memorial Foundation Award

Societies:

American Society for Microbiology
American Association for the Advancement of Science
Society for Experimental Biology and Medicine
American Association for Cancer Research, Inc.
American Society for Virology, Inc.

Memberships and Affiliations:

1975-1978 Member, Viral Cancer Program Coordinating Committee
1975-1976 Ad Hoc Member, Experimental Virology Study Section, NIH
1975-1978 Member, Viral Oncology Scientific Advisory Committee for FCRC
1976-1980 Member, Experimental Virology Study Section, NIH
1977- Member, Editorial Board, International Journal of Cancer
1977-1986 Associate Editor, Journal of the National Cancer Institute
1980-1985 Editorial Advisory Board, Biochimica et Biophysica Acta (BBA Reviews on Cancer)
1981- Associate Editor, Cancer Research
1983- Executive Committee, Duke Comprehensive Center, Duke University Medical Center
1984 Mott Selection Committee, General Motors Cancer Research Foundation
1984- Advisory Committee, Maimonides Conferences on Cancer Research
1984-1990 Editorial Board, Virus Research
1984-1987 Scientific Advisory Committee, American Cancer Society
1985-1987 External Scientific Review Committee, Comprehensive Center, The University of Alabama in Birmingham
1985- Editorial Advisory Board, Cancer and Metastasis Reviews
1985- Editorial Board, Cancer Reviews
1985-1989 Councillor, Society for Experimental Biology and Medicine
1985-1990 Extramural Advisory Board, Cancer Center, The University of Arizona
1986 Program Chairman, American Association of Cancer Research
1986 Co-organizer, Princess Takamatsu Symposium
1986- Guest Editor, Japanese Journal of Cancer Research (Gann)
1986- Editorial Board, Environmental and Occupational Health Sciences
1986-1987 Member, Advisory Committee, American Type Culture Collection
1987-1989 Editorial Advisory Board, Molecular Endocrinology
1987- Editorial Board, Oncogene
1988-1989 Advisory Editorial Board, ISI Atlas of Science: Biochemistry
1988- Member, Blood Services Scientific Council, American Red Cross
1989-1991 Editorial Board, Cancer Communications

1989-1992 Editorial Board, The New Biologist
1989 Visiting Professor, University of Texas, San Antonio
1990- Advisory Board, BBA Reviews on Cancer, Biochimica et Biophysica Acta
1990- General Motors Visiting Professor, University of Wisconsin-Madison Medical School
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Research Interests:

Molecular genetics of cancer; retrovirology; cellular growth regulation by growth factors and their receptors.

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COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF: Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Susan Power of Cell & Molecular Technologies, Inc., Phillipsburg, New Jersey, United States of America, declare as follows:

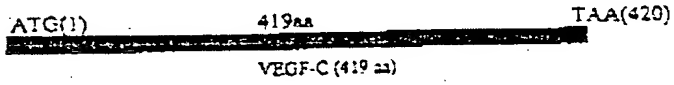
1. I am currently a Senior Molecular Biologist for Cell & Molecular Technologies, Phillipsburg, New Jersey. I have held this position since July, 1999. Prior to that appointment I was a post-doctorate research scientist, studying the molecular biology of retenoids and their role in limb bud development, at the University of Wisconsin, Madison, Wisconsin for two years. Prior to that appointment, I was a post-doctorate research scientist, studying the molecular biology of the transcription factor vHNF1 at the Pasteur Institute in Paris, France for four years. I received my Ph.D. in 1991 in Microbiology, from the National University of Ireland, Galway, Ireland.

2. The Patent Attorneys for Human Genome Sciences (HGS) requested that I perform the following experiments in order to determine whether the 350 amino acid form of VEGF-2 (corresponding to residues 70 to 419 of the 419 amino acid form of VEGF-2) fused in frame with a heterologous signal sequence would result in the expression and secretion of the protein from eukaryotic cells. They have also requested that all experiments that I conducted employ techniques routinely available by March, 1994. I have done this and the experiments I have conducted are described herein. Unless I state otherwise, all methods used herein were available prior to March, 1994.

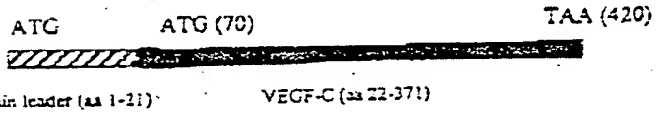
3. To determine whether the 350 amino acid form of VEGF-2 could be secreted from cells when attached to a heterologous signal sequence, I transfected eukaryotic cells with expression vectors encoding the 419 amino acid form of VEGF-2, or the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence. I grew the transfected cells under conditions to allow the cells to express the gene products encoded by the vectors. At various time points, I collected both cell lysates and culture medium and assayed for the presence of VEGF-2, in order to determine if the cells were secreting VEGF-2. The presence of VEGF-2 in either the cell lysates or culture medium was determined by Western Blot analysis of the samples using a polyclonal antibody to VEGF-2, which recognizes both the precursor form and the processed form of VEGF-2

4. The design of the expression vectors used in the study is as follows:

419 amino acid form of VEGF-2 (followed by a STOP codon at position 420):



350amino acid form of VEGF-2 linked to a heterologous signal sequence (followed by a STOP codon at position 420):



5. The nucleotide sequences encoding the 419 and the 350 amino acid forms of VEGF-2 were obtained directly from the American Type Culture Collection (ATCC). ATCC Deposit No. 97149 contains the nucleotide sequence encoding the 419 amino acid form of VEGF-2. ATCC Deposit No. 75698 contains the nucleotide sequence encoding the 350 amino acid form of VEGF-2. The nucleotide sequence encoding the 419 amino acid form of VEGF-2

was engineered to be flanked by an Eco RI site at the 5'end and a Not I site at its 3'end. The second construct contained the nucleotide sequence encoding the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, Ig k-chain leader signal sequence, a recognized signal sequence which was available as of March, 1994, and was engineered to contain a Bam HI site at its 5'end and a Not I site at its 3' end. The sequence of each of these constructs was confirmed to be correct and is detailed in Appendix I, attached hereto.

6. Each VEGF-2 construct was subcloned into the expression vector pCMV-I (see attached plasmid map, attached hereto as Appendix II), so that the VEGF-2 sequences were under the control of a CMV-I promoter, a promoter routinely used as of March, 1994. The 419 amino acid form of VEGF-2 was subcloned into the Eco RI/Not I sites of pCMV-I, while the 350 amino acid form of VEGF-2 fused in frame with a heterologous signal sequence, was subcloned into the Bam HI/Not I sites of pCMV-I (see plasmid map, Appendix II).

7. The two VEGF-2 constructs were transiently transfected in duplicate, using the lipofectin method, comparable methods were routinely used as of March, 1994, into the Human Embryonic Kidney cell line, HEK-293 tsA-0, a cell line which was also routinely used as of March, 1994. As a control for transfection efficiency, each construct was co-transfected with the vector pCMV-β-gal. The efficiency of transfection was determined by β-gal staining 48 hours after transfection. As a negative control the vector pCMV-I without an insert was transfected in parallel.

8. The transfection design is as follows:
10 dishes transfected with: pCMV-I-VEGF-419;
10 dishes transfected with: pCMV-I-signal sequence-VEGF-350;
10 dishes transfected: pCMV-I ;
2 dishes transfected with: pCMV-I-VEGF-419 + pCMV-β-gal; and
2 dishes transfected with: pCMV-I-signal sequence-VEGF-350 + pCMV-β-gal.

9. After transfection, DMEM medium containing 3% serum was added to the cells. Aliquots of cell extracts and conditioned medium were prepared from each transfection at: T_0 hours, T_{16} hours, T_{24} hours, T_{48} hours and T_{72} hours, in duplicate.

10. At the time of harvesting the cells and medium were treated as follows:
Medium: Harvested medium was concentrated 3 fold using Centricon 10 concentrator devices. One volume of 2 x PAGE loading dye was added to each sample.
Cell Extracts: The cells were harvested by trypsinization and collected by centrifugation. The cell pellet was resuspended and lysed in 250µl of 1x PAGE loading buffer.

11. To determine the transfection efficiency, dishes transfected with the pCMV-β-gal construct were fixed and stained for β-gal activity 48 hours after transfection. All dishes were found to have the same percentage of transfected cells (70%).

12. Each protein sample was subjected to Western analysis as outlined below. Prior to loading on to a 12% (w/v) Tris-Glycine SDS-Polyacrylamide gel, the samples were boiled for 5 minutes and cooled on ice. The two end lanes of each gel contained the appropriately sized molecular weight markers to estimate the migration rate of proteins predicted to run in the 16 to 85 kDa size range. The samples were electrophoresed according to standard conditions.

13. Following electrophoresis, the samples were transferred to a PVDF membrane. Each membrane was blocked by a one hour incubation in phosphate buffered saline (PBS) containing 3% Bovine Serum Albumin (BSA). The blot was then incubated at 4°C in PBS containing 0.1% BSA and 500ng/ul of purified rabbit anti-VEGF-2 antibody, a polyclonal antibody which recognizes the precursor form and the processed form of VEGF-2. After three 5 minute washes in PBS containing 0.1% Tween, the blot was incubated for 1 hour in PBS containing 0.1% BSA and a 1:3000 dilution of Goat Anti-Rabbit IgG

Horse Radish Peroxidase (HRP) conjugated antibody. The blot was washed three times for 5 minutes in PBS containing 0.1% BSA. The blot was developed with 2ml/blot of ECL detection reagent (obtained from Amersham) for one minute and then exposed directly to Polaroid films for approximately 5 seconds.

14. The result of the experiment is shown in Figure 1, attached hereto. The samples included in the figure are as follows:

Immunoblot analysis of VEGF derivatives transiently expressed in HEK293T cells

Lane	Pellet/Supernatant	350aa -signal / 419aa	T (h) post-transfection
Gel 1			
1	P	419	24
2	S	419	24
3	P	350-signal	24
4	S	350-signal	24
5	P	350-signal	24
6	S	350-signal	24
7	P	negative control	24
8	S	positive control	48
Gel 2			
9	P	419	48
10	S	419	48
11	P	419	48
12	S	419	48
13	P	350-signal	48
14	S	350-signal	48

15	P	350-signal	48
16	S	350-signal	48
17	P	negative control	
18	S	negative control	
Gel 3			
19	P	419	72
20	S	419	72
21	P	419	72
22	S	419	72
23	P	350-signal	72
24	S	350-signal	72
25	S	350-signal	72
26	P	350-signal	72

15. The Western Blot analysis indicates that a doublet of approximately 30kDa was present in the medium collected from the transfection of both the 419 amino acid form of VEGF-2 and the 350 amino acid-VEGF-2 signal sequence constructs (see Figure 1). The secreted protein was visible beginning at 16 hours after transfection. The secreted product from cells containing the 419 amino acid construct and the 350 amino acid-VEGF2 signal sequence construct are approximately the same size.

AND I declare that all the statements made in this Declaration are of my own are true in every particular, and that all statements made on information and belief are believed to be true.

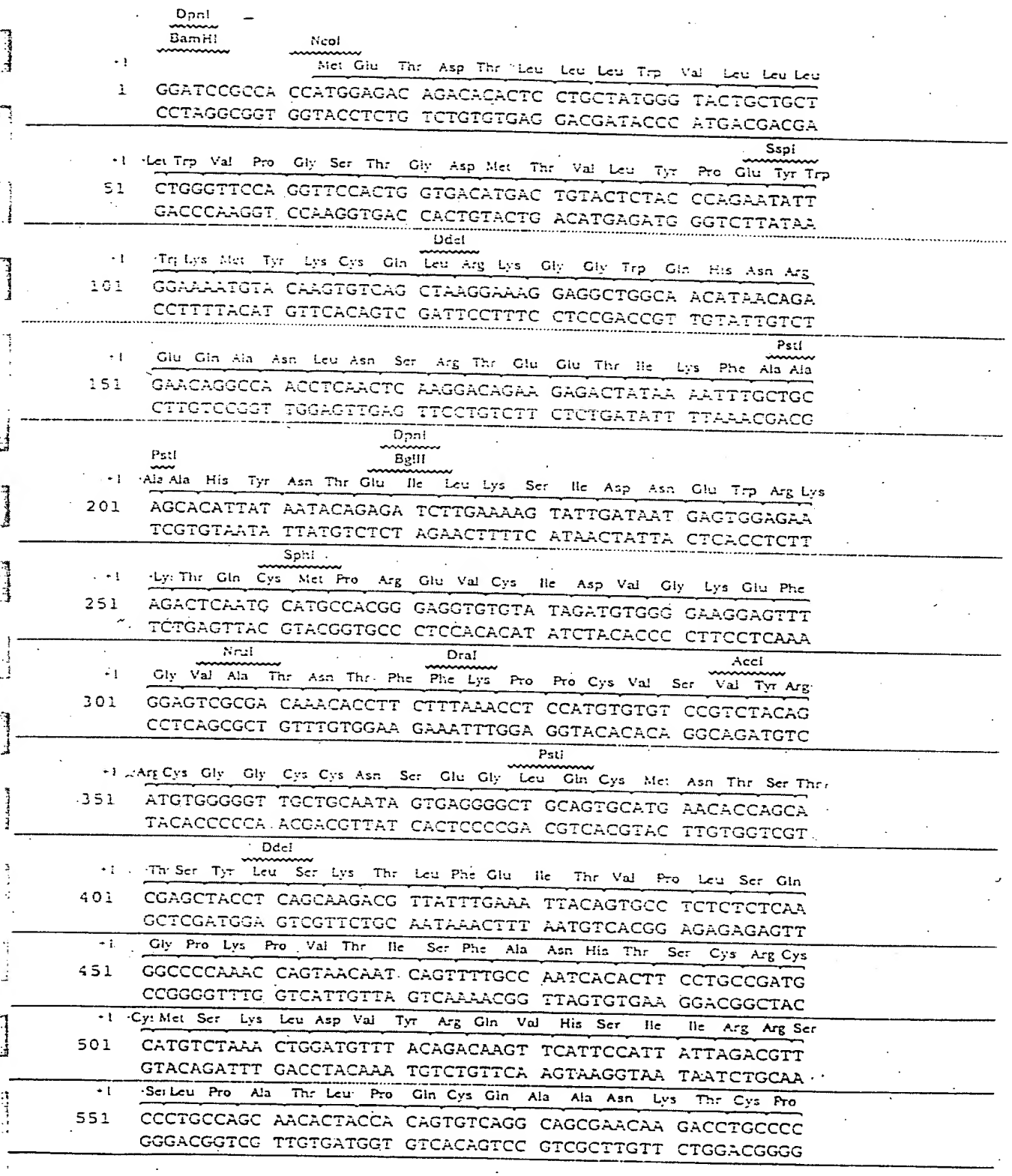
Sworn by the said Susan Power, Susan Power at Phillipsburg, New Jersey, on this 13th day of December 2000; before me Maryann White.

Notary Public

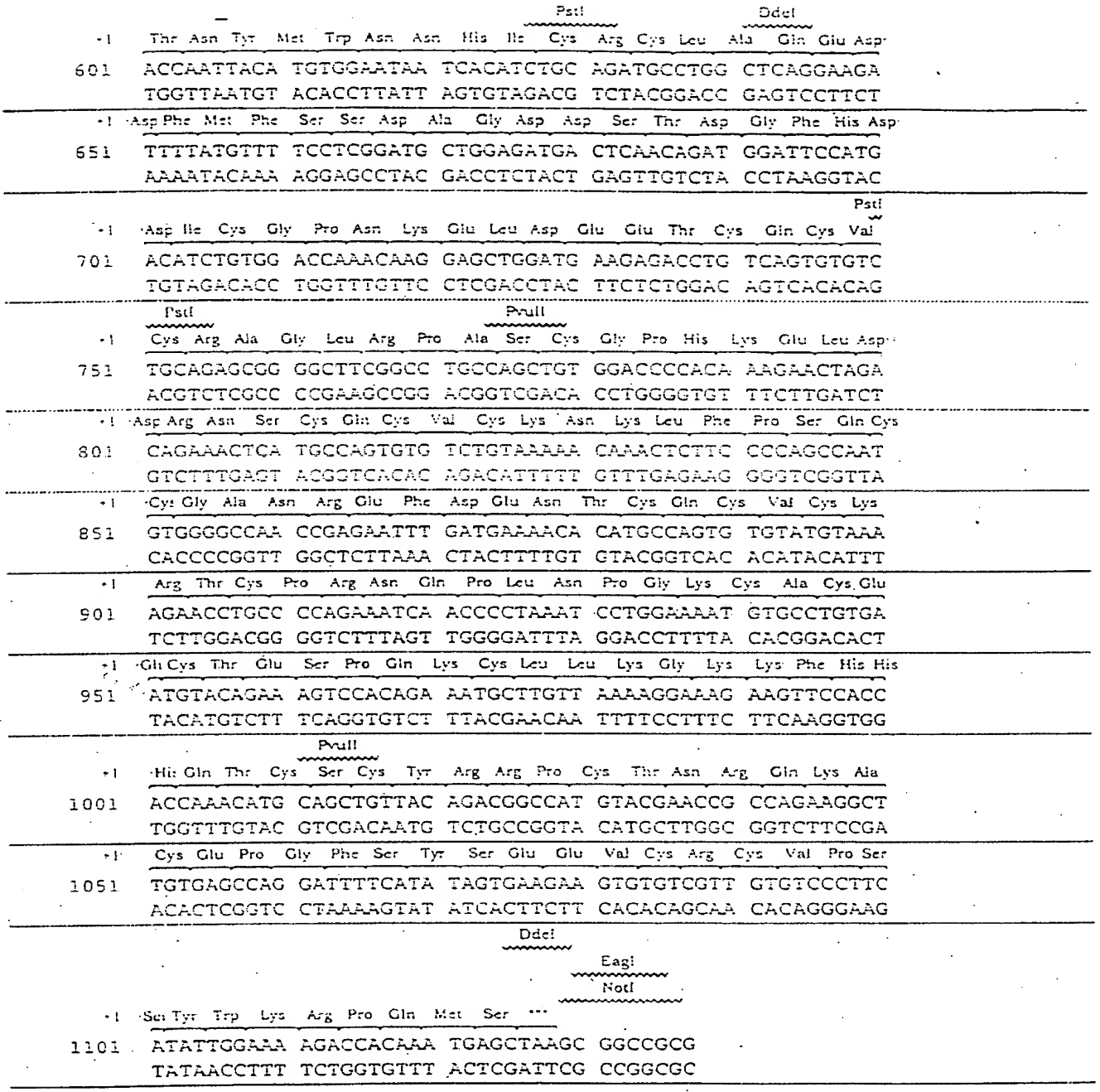
MARYANN WHITE
NOTARY PUBLIC, State of New York
No. 4883761
Qualified in Nassau County
Certification Filed in New York County
Commission Expires January 26, 2001

** TOTAL PAGE.07 **

VEGF-2 350aa +Signal Sequence:



Appendix I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PstI
+1 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val
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TGTAGACACC TGGTTTGTTC CTCGACCTAC TTCTCTGGAC AGTCACACAG
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EagI
NotI
+1 Ser Tyr Trp Lys Arg Pro Gln Met Ser ***
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VEGF-2 419aa Sequence:

```
        EcoRI
+1                                    Met His  Leu  Leu Gly Phe  Phe  Ser Val Ala
1       GAATTCGTGG GTCCTTCCAC CATGCACTTG CTGGGCTTCT TCTCTGTGGC
        CTTAAGCACC CAGGAAGGTG GTACGTGAAC GACCCGAAGA AGAGACACCG

                                          SmaI
                                          XmaI
                                          AvaI        NruI
+1  Ala Cys Ser Leu Leu Ala Ala Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala
51      GTGTTCTCTG CTCGCCGCTG CGCTGCTCCC GGGTCCTCGC GAGGCGCCCG
        CACAAGAGAC GAGCGGCGAC GCGACGAGGG CCCAGGAGCG CTCCGCGGGC

+1  Ala Ala Ala Ala Ala Phe Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro
101     CCGCCGCCGC CGCCTTCGAG TCCGGACTCG ACCTCTCGGA CGCGGAGCCC
        GGCGGCGGCG GCGGAAGCTC AGGCCTGAGC TGGAGAGCCT GCGCCTCGGG

                                                DpnI
                                                BglII
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151     GACGCGGGCG AGGCCACGGC TTATGCAAGC AAAGATCTGG AGGAGCAGTT
        CTGCGCCCGC TCCGGTGCCG AATACGTTCG TTTCTAGACC TCCTCGTCAA

                                                                  SspI
+1  Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr
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        TGCCAGACAC AGGTCACATC TACTTGAGTA CTGACATGAG ATGGGTCTTA

    SspI                      DdeI
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                                                                  PstI
+1  Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala
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                              DpnI
    PstI                      BglII
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                    SphI
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            NruI                  DraI                            AccI
+1  Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
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    AccI                                    PstI
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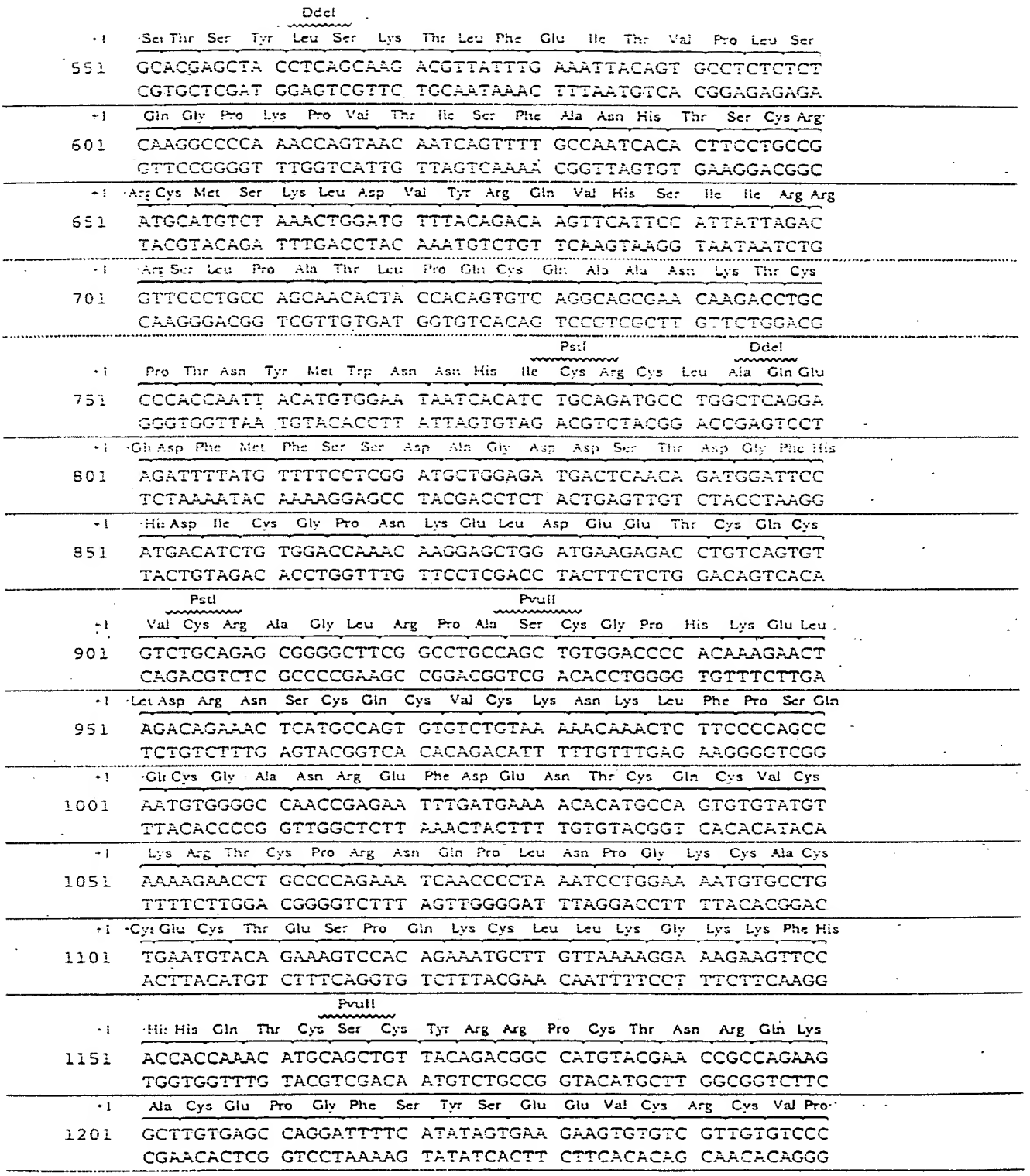
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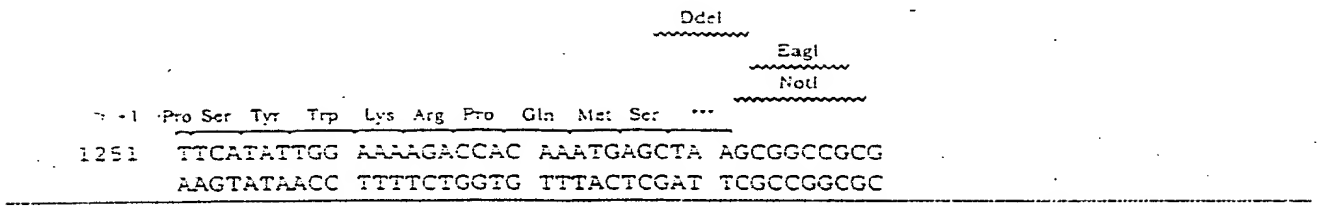
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EagI
NotI
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AAGTATAACC TTTTCTGGTG TTTACTCGAT TCGCCGGCGC

STRAIN SHEET

STRAIN CMV I STORAGE NUMBER 11:64

PEDIGREE:

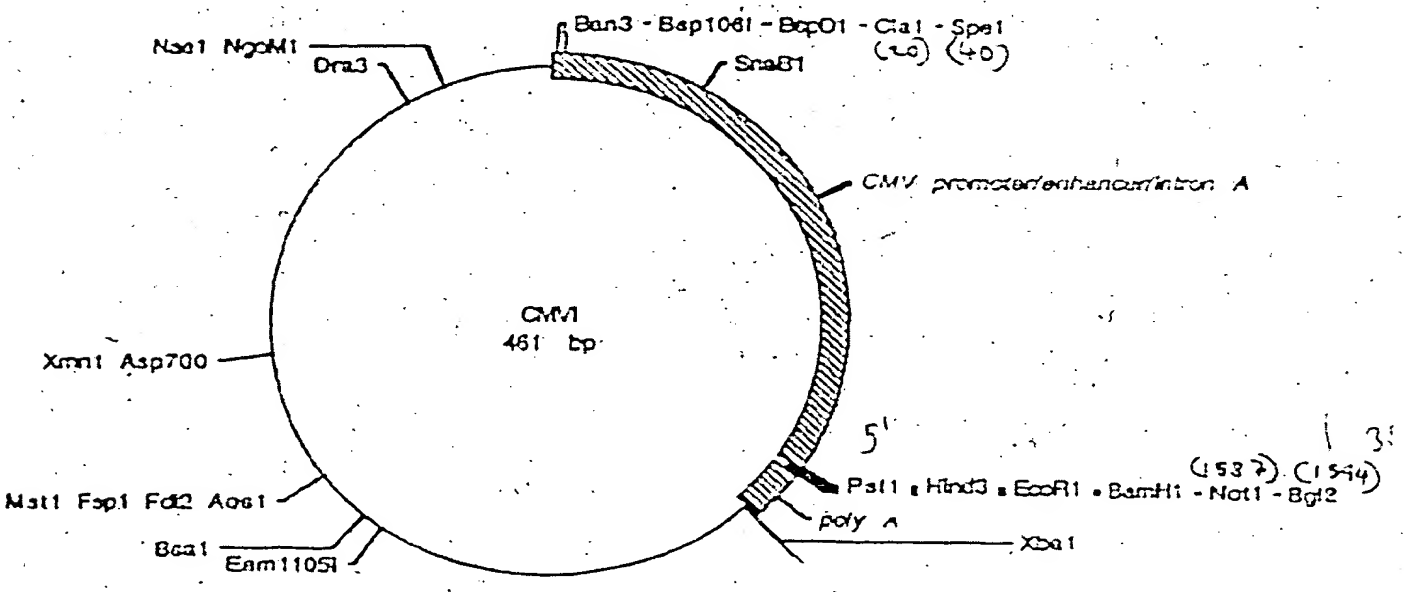
-CMVI was constructed in the pSV7 (nee pSG5 from Stratagene, with an expanded polylinker) backbone by replacing the SV40 promoter from pSV7 with the CMV promoter/enhancer/intron, via 5' Sal I/Xho I (sites destroyed) and 3' Hind III.

-CMVI is 4613 bp.

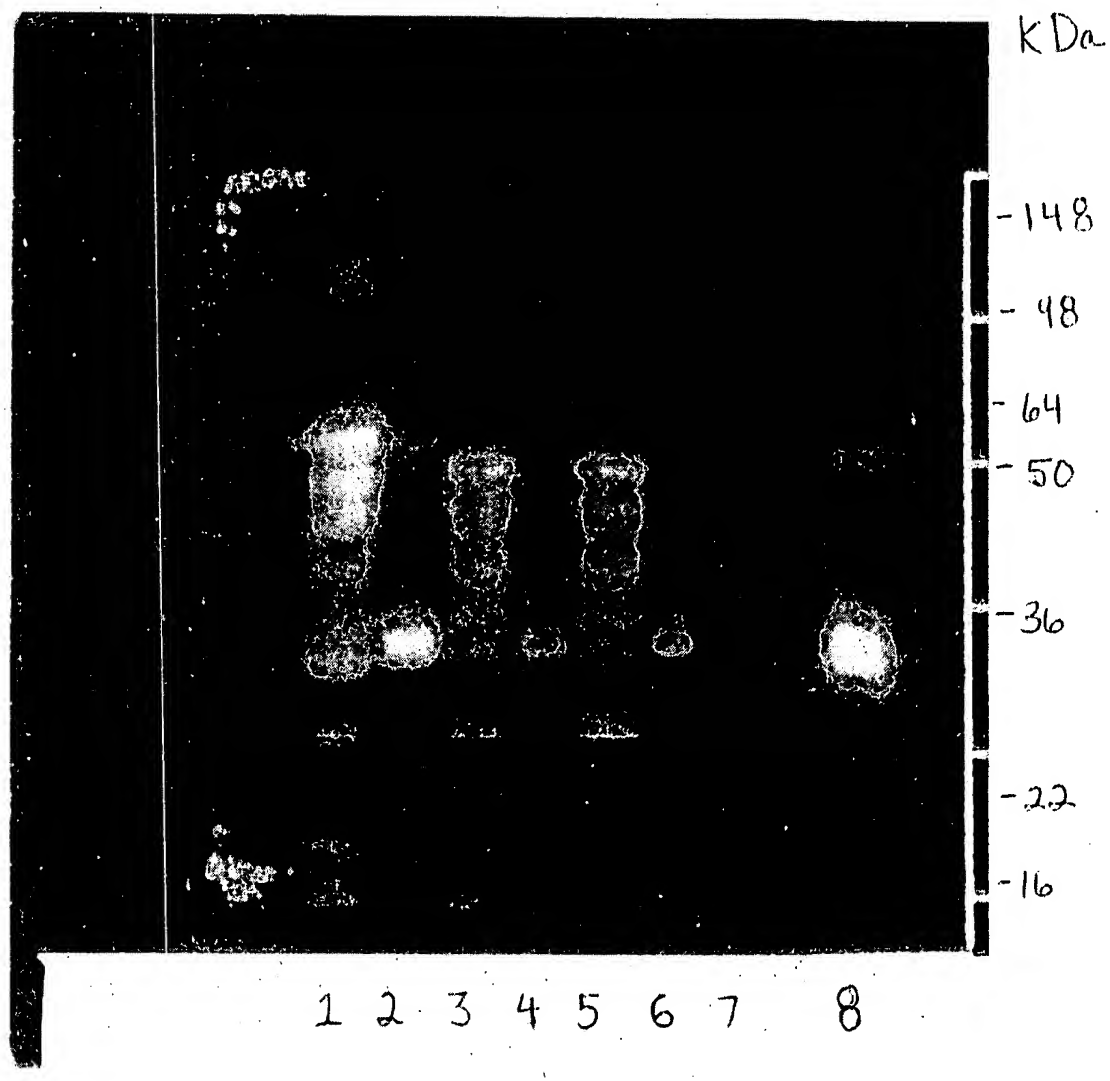
-CMVI uses ampicillin resistance.

-CMV promoter/enhancer + Intron A: nt #'s 1-1566.
-Polylinker: nt #'s 1566-1597 (5'- Hind III - Eco RI - Bam HI - Not I - Bgl II - 3').
-SV40 polyA addition sequence: nt #'s 1598-1745.
-If for some reason you want to remove the SV40 polyA addition sequence, you can cut with either Sal I or Xba I (these 2 sites border the 3' end of this sequence).

GROWTH REQUIREMENTS:

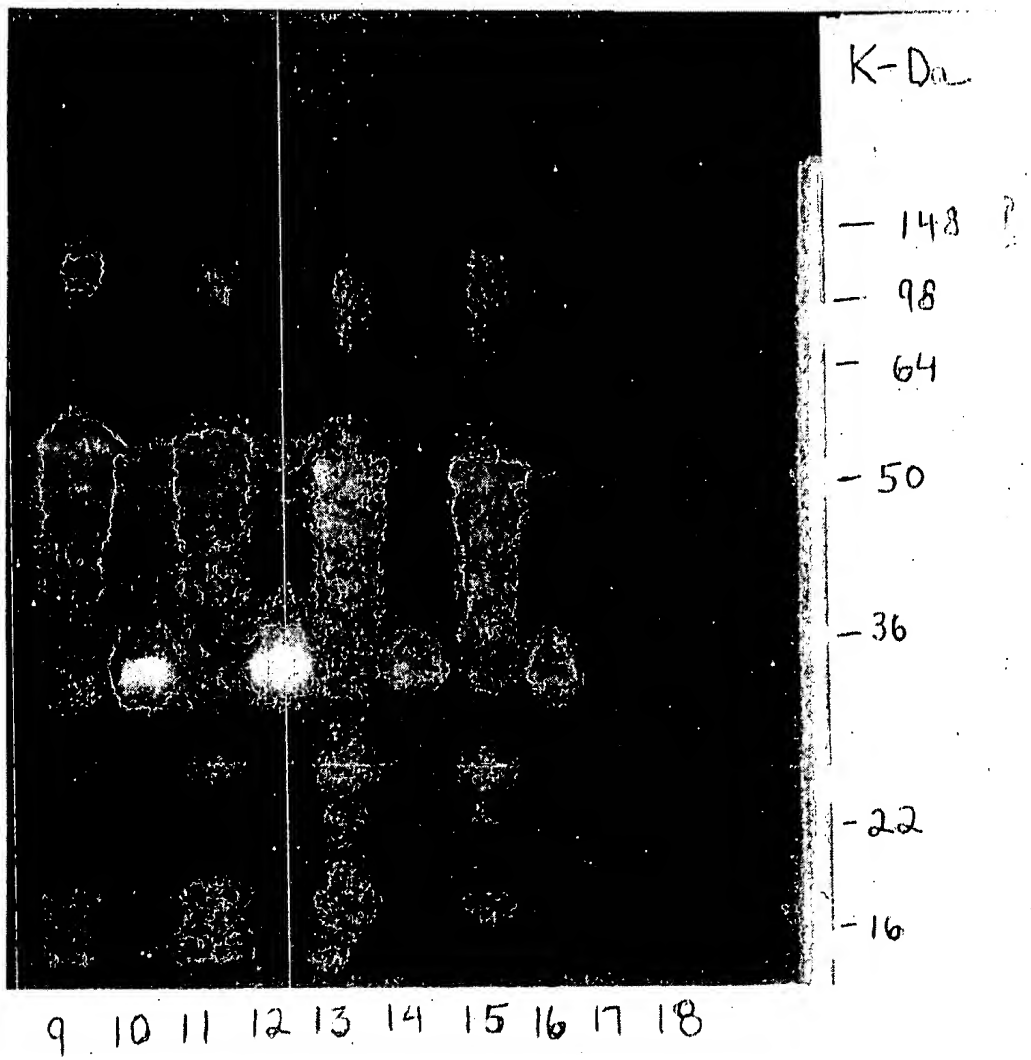


Appendix II

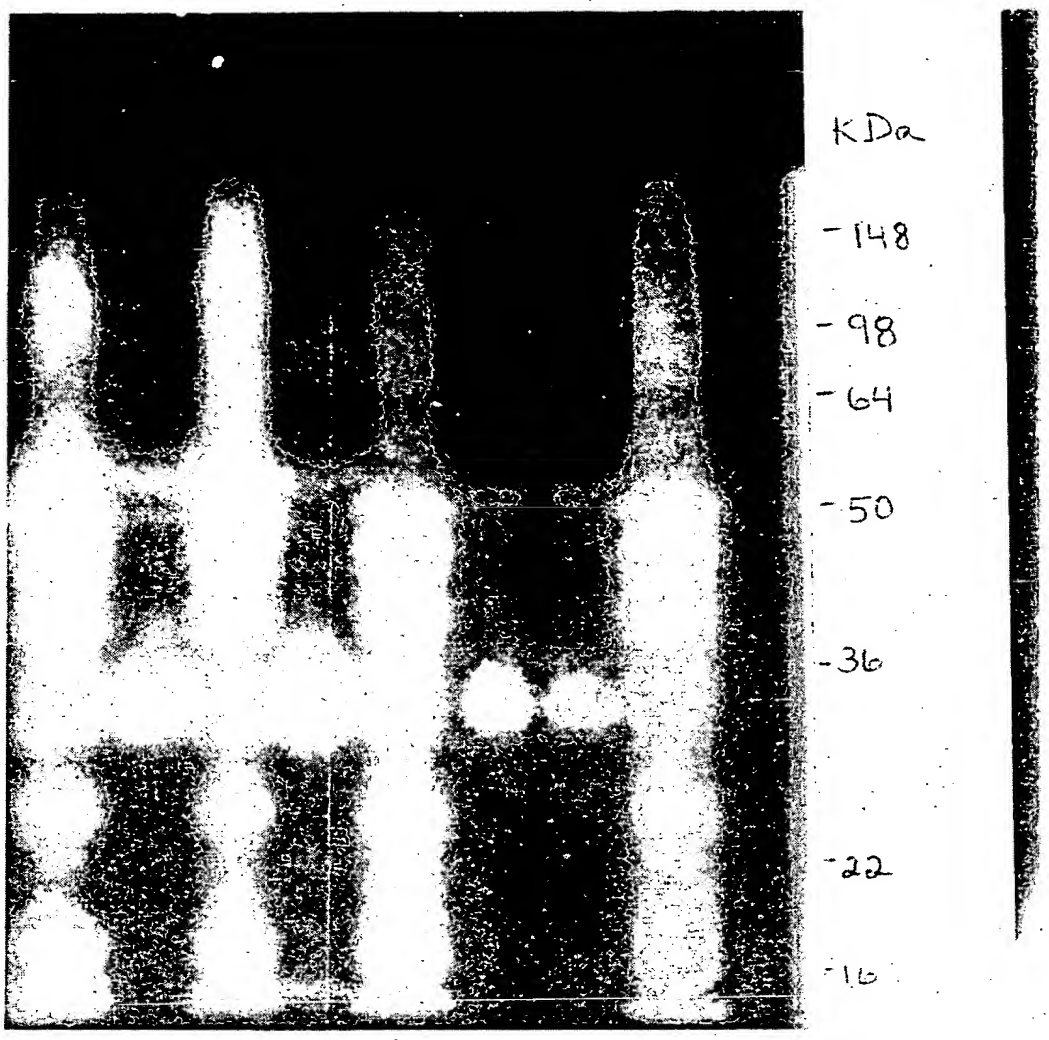


Power Declaration

Figure 1 Gel 1



Power Declaration
Figure 1 - Gel 2



Power Declaration
Figure 1 - Gel 3